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CD3-CD4+ lymphoid variant of hypereosinophilic syndrome: nodal and extranodal histopathological and immunophenotypic features of a peripheral indolent clonal T-cell lymphoproliferative disorder

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Running heads: The clonal T-cell disease in CD3-CD4+ L-HES

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ABSTRACT

The CD3-CD4+ lymphoid variant of hypereosinophilic hyndrome is characterized by hypereosinophilia and clonal circulating CD3-CD4+ T-cells. Peripheral T-cell lymphoma have been described during this disease course, and we observed in our cohort of 23 patients two cases of angio-immunoblastic T-cell lymphoma. We focus here on histopathological (n=12 patients) and immunophenotypic (n=15) characteristics of CD3-CD4+ lymphoid variant of hypereosinophilic syndrome. Atypical CD4+ T-cells lymphoid infiltrates were found in ten of twelve CD3-CD4+ L-HES patients, in lymph nodes (n=4/4 patients), in skin (n=9/9) and other extra-nodal tissues (gut, lacrymal gland, synovium). Lymph nodes displayed infiltrates limited to the interfollicular areas or even an effacement of nodal architecture, associated with proliferation of arborizing high endothelial venules and increased follicular dendritic cell meshwork. Analysis of two fresh skin samples confirmed the presence of CD3-CD4+ T-cells. Clonal T-cells were detected in at least one tissue in eight patients, including lymph nodes (n=4/4): the same clonal T-cells were detected in blood and in at least one biopsy, with a maximum delay of 23 years between samples. In the majority of cases, circulating CD3-CD4+ T-cells were CD2hi (n=9/14), CD5hi (n=12/14), and CD7- (n=4/14) or CD7low (n=10/14). Angio-immunoblastic T-cell lymphoma can also present with CD3-CD4+ T-cells: despite other common histopathological and immunophenotypic features, CD10 expression and follicular helper T-cells markers were not detected in lymphoid variant of hypereosinophilic syndrome patients, except in both patients who developed angio-immunoblastic T-cell lymphoma, and only at T-cell lymphoma diagnosis. Taken together, persistence of tissular clonal T-cells and histopathological features define CD3-CD4+ lymphoid variant of hypereosinophilic syndrome as a peripheral indolent clonal T-cell lymphoproliferative disorder, which should not be confused with angio-immunoblastic T-cell lymphoma.

Introduction

Hypereosinophilic syndromes (HES) are defined by a blood hypereosinophilia (HE) > 1.5 G/L on two examinations (and/or tissue HE), organ damage (and/or dysfunction attributable to tissue HE) and exclusion of other explanations for organ dysfunction.[1] The lymphoid variant of HES (L-HES) is considered as a

reactive HES characterized by the presence of an abnormal circulating T-cell subset, with a Th2 profile and able to produce eosinophilopoietic cytokines such as interleukin-5.[1,2] The best characterized is the CD3-CD4+ aberrant phenotype with 38 cases cited to date,[3–12] and 21 more patients recently reported by the French Eosinophil Network.[13]

Circulating and/or nodal CD3-CD4+ or CD3lowCD4+ T-cells have also been detected by flow cytometry in various peripheral T-cell lymphomas (PTCL) but mainly in angioimmunoblastic T-cell lymphoma (AITL).[14–18] PTCLs have also been reported during CD3-CD4+ L-HES course.[6,9,19–24] Two of 23 patients currently followed in the French Eosinophil Network, and one more patient recently reported by others,[25] developed well-defined AITL several years after L-HES diagnosis, which thus raised the problem of the diagnosis of well-defined T-cell lymphoma in patients who have clonal circulating T-cells.

In this study, we focused on the lymphoid infiltrates in lymph nodes, skin and other available biopsies of tissue involved in L-HES, to assess the presence of clonal T-cells at diagnosis and during CD3-CD4+ L-HES course. We secondly aimed to distinguish L-HES from AITL by comparing histopathological and immunophenotypic characteristics between both entities.

Methods

Patients

Twenty-three hypereosinophilic syndrome (HES) patients with a documented presence of CD3-CD4+ aberrant subset and a negative FIP1L1-PDGFRA fusion gene research are currently followed in the French Eosinophil Network. For the present study, 16 patients (P1-P16) were included, 12 of them had available tissue biopsies during L-HES course. All satisfied criteria for HES (n=15) or hypereosinophilia (HE) (n=1, P13, no organ damage or clinical manifestation) criteria in accordance with the latest updated consensus definitions.[1] Main clinical characteristics are summarized in Table 1. For the seven remaining patients, no complementary lymphocyte immunophenotyping was performed, no biopsy was performed (n=5) or biopsies were not available for analysis (n=2). The study was approved by the Lille Hospital Ethical Committee and carried out in accordance with the Helsinki convention.

Ten of these patients had bone marrow biopsies at CD3-CD4+ L-HES diagnosis in order to exclude a T-cell lymphoma (P2-4, P8, P9, P11, P12, P14-16, not shown).

Four patients had lymph nodes biopsies for a suspicion of T-cell lymphoma during follow-up (Patient P1, P3, P4 and P10). For this work, all their biopsies were retrieved for further explorations and a centralized compared analysis.

Finally, patient P4 and P16 developed a well-defined AITL during L-HES course (AITL/L-HES patients):

Patient P4. Patient P4 was 18 years-old when CD3-CD4+ L-HES diagnosis was made in 1999 and was previously reported by us.[26] He presented with eczema-like lesions, rare episodes of angioedema and multiple adenopathy. Despite high circulating CD3-CD4+ T-cells count (28 G/L), lymph nodes histological examination concluded to lymphoid reactive hyperplasia: as he was in really good health status and slightly embarrassed by his symptoms, diagnosis of T-cell lymphoma was not retained and he did not receive any treatment, excepted topical corticosteroids for eczema. He was lost to follow-up from 2004 to 2011. He was treated with high-dose corticosteroids for the first time in 2013 for a severe generalized eczema, polyarthritis and high HE (25 G/L). AITL diagnosis was made in July 2014 after he was hospitalized for fever, weight loss, erythroderma, multiple liver lesions and adenopathy. Eighteen-fluoro-deoxyglucose positron emission tomography (18-FDG-PET) scan showed a generalized lymphadenopathy. Lymphoma progressed rapidly despite chemotherapy with a severe lung involvement and the patient died ten weeks after AITL diagnosis.

Patient P16. First L-HES symptoms were pruritus and/or urticarial plaques, polyarthritis and occurred in 2002. L-HES diagnosis was made in 2004 on the association of HE (4.5 G/L), clonal TCRγ rearrangement and really small but persistent CD3-CD4+ circulating T-cells subset. Corticosteroids and interferon-alpha given as a corticosteroid-sparing treatment have permit to improved symptoms and normalized eosinophils count. All treatments were stopped in October 2009. In July 2010, diagnosis of AITL was made after an amoxicillin-

induced rash, followed by systemic manifestations including fever, lips angioedema and polyarthritis. AITL has never been controlled despite appropriate chemotherapy (alemtuzumab and CHOP chemotherapy, vinblastine) and he died 4 months after AITL diagnosis.

Histology and Immunohistochemistry

All available biopsies in CD3-CD4+ L-HES patients were retrieved for new analysis by an experienced pathologist (MCC) and further explorations were carried out similar to explorations performed for T-cell lymphomas. Nine patients had at least one available skin biopsy during their follow-up, four patients had at least one lymph node biopsy for a suspicion of lymphoma, five had digestive biopsies (available in four patients), one patient had a synovial biopsy, one patient had a lacrymal gland biopsy (available) and biopsy of both parotids (unavailable). All available biopsies are summarized in Table 1. Two patients who developed AITL during L-HES course (AITL/L-HES) had bone marrow, skin (P4 and P16) and lymph node biopsies (P4) at AITL diagnosis.

Briefly, morphological analysis of skin, lymph node and other tissue biopsies focused on description of atypical lymphoid cells, pattern of infiltration and presence of other cellular types in the infiltrates (eosinophils and plasmocytes notably). Deparaffinized tissue sections were stained for CD3, CD4, CD5, CD8, CD10, CD20, CD56, PD1, CXCL13. In situ hybridization for EBV was performed on deparaffinized tissue sections using a FITC coupled specific peptidic nucleic acid probe allowing recognition of EBER transcripts. T-cell receptor gamma (TCRγ) rearrangement analysis was performed in tissues as previously described.[27] Two fresh skin samples were comminuted with a razor blade, digested by collagenase and analyzed by flow cytometry after multiple washes using fluorochrome-coupled anti-CD3, -CD4, -CD8, -CD45 antibodies.

Multiparameter flow cytometry and TCRγrearrangement study in circulating CD3-CD4+ T-cells

All CD3-CD4+ L-HES patients followed in the French Eosinophil Network had been diagnosed on the basis of circulating CD3-CD4+ T-cell aberrant subset detected by flow cytometry as previously described.[13] For this work, fifteen were newly collected (n=13), or reanalyzed (n=2) with Kaluza® 1.2 software (Beckman Coulter). Fluorochrome-coupled antibodies (CD45, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CXCL13, and PD1) were purchased from Beckman Coulter®. A CD3-CD4+ subset was considered to be positive or negative for a surface marker if > 95% or < 5% of the cells expressed or not the marker, respectively. When only a part of the subset expressed the surface marker, the percentages of positive cells were noted. Mean fluorescence intensities of pan T-cell markers (CD2, CD5, CD7) on CD3-CD4+ T-cells were expressed as “low” or “high” in comparison to CD3+CD4+ T-cells. Study of CD10 expression, which was not done routinely before, was performed on the 14 newly obtained samples. TCRγ rearrangement analysis was also performed in peripheral blood as previously described.[27] Analysis was performed with the 3130 Genetic Analyzer® and the GeneMapper 4.1® software from Applied Biosystems®.

Results

Histopathological characteristics in lymph nodes of CD3-CD4+ L-HES patients mimic AITL

We assessed the lymphoid infiltration in the lymph nodes of four CD3-CD4+ L-HES patients (P1, P3, P4, P10). Lymph node biopsies were performed for a suspicion of lymphoma because of multiple adenopathy although the patients kept a good general status and did not develop any general symptoms (including patient P4 who had biopsies in 2000 and 2011: histopathological characteristics at AITL diagnosis in 2014 are described below). In all cases, eosinophils and lymphoid infiltrates were observed when analyzed in routine practice, without any diagnosis of lymphoma, except for patient P3: AITL diagnosis was evoked but was retrospectively excluded according to the 2008 WHO classification.

The main histopathological features were lymphoid infiltrates, with various extensions, from limited distribution with dense interfollicular infiltrates to effacement of lymph node architecture, sparing the peripheral cortical sinuses (Table 2, Figure S1). Lymphoid infiltrates were composed of atypical monomorphic small to medium size lymphoid cells, with irregular nucleus and scarce cytoplasm. Other typical features were proliferation of arborizing high endothelial venules (HEV), increased CD23+ follicular dendritic cell meshwork. In all cases, lymphoid cells were CD3+CD4+CD5+ T-cells but without expression of CD10 or TFH markers (CXCL13, PD1). There were no EBV-positive B-cells. A clonal TCRγ rearrangement was detected in all patients (Table 2). In one case (P10), circulating CD3-CD4+ T-cell subset dramatically increased at lymphoma suspicion: after lymphoma diagnosis was excluded, corticosteroids alone allowed a remarkable decrease of CD3-CD4+ T-cells and an 18-FDG-PET scan showed decrease of lymph nodes (Figure S2). Afterward, the patient has been transiently treated with ciclosporin A for a severe psoriasis, and kept in good health-status for 3 years under corticosteroids alone (prednisone 10mg/d).

Skin histopathology in CD3-CD4+ L-HES

A superficial and/or deep dermis inflammatory infiltrate without epidermotropism was observed in all nine cases. No patient had epidermis involvement and two patients had a hypodermis infiltration (Table 3). Infiltrates were composed of eosinophils, rare plasma cells and the same atypical lymphoid cells as those observed in lymph nodes. IHC revealed that all these lymphoid cells were also CD3+CD4+CD5+ T-cells (Figure 1A) but without any expression of CD10 or TFH markers (CXCL13, PD1). The same lymphoid infiltrates were observed in all available successive skin biopsies for a given patient (n=6/6) (Figure S3).
In L-HES, CD3 is detectable in cytoplasm of permeabilized CD3-CD4+ T-cells by flow cytometry.[3] Since IHC does not distinguish membranous from cytoplasmic CD3 expression in paraffin section, two fresh skin samples (P1 and P2) were analyzed by flow cytometry: in both cases, a large majority of CD4+ T-cells isolated in the skin sample had no membranous CD3 expression (Figure 1B). TCRγ rearrangement study performed at the same time in peripheral blood and skin confirmed the presence of the same clonal T-cells in P1 and P2 (Figure 1C). In the whole population, a clonal TCRγ rearrangement was detected in at least one

skin biopsy in 6/9 patients. Altogether, these results show that besides eosinophils, CD3-CD4+ clonal T cells are also present in inflammatory infiltrates in skin lesions in CD3-CD4+ L-HES.

Other extra-nodal tissues histopathology in CD3-CD4+ L-HES

Digestive biopsies were available in four patients (P5, P10, P11 and P14). Eosinophil infiltration was observed in patients P10, P11 and P14, but was particularly high in patient P10, in association with lymphoid infiltrates which partially destructed colic glands: lymphoid cells had the same characteristics as previously and IHC also found CD3+CD4+CD5+ T-cells, which did not express CD10, CXCL13 or PD1. CD8+ T-cells and CD20+ B-cells were rare. The same characteristics were found in a synovial biopsy (P5) and in a lacrymal gland biopsy (P1) performed for a "Kimura like" disease (Figure S4). In all 3 biopsies with lymphoid infiltrates, a clonal TCRγ rearrangement was detected (Table 4).

Immunophenotypic characteristics of circulating CD3-CD4+ T-cells in L-HES

We have shown above that aberrant T-cells had the same immunophenotype CD3+CD4+CD5+ but did not express CD10 or TFH markers CXCL13 and PD1 in all tissues. We studied peripheral blood samples to characterize circulating CD3-CD4+ T-cells in L-HES. Aberrant T-cells presented with non-specific cytological abnormalities (Figure S5). In comparison to conventional CD3+CD4+ T-cells, CD2 and CD5 expressions were identical or increased, CD7 expression was absent (n=4/14) or partial/diminished (n=10/14). CD10 expression was never detected (n=0/14) (Figure S6, Supplementary Table S1).

Persistence of the same clonal T-cells in blood and various tissues samples collected during CD3-CD4+ L-HES course

As we found that the lymphoid infiltrates were similar in all successive biopsies for a given patient, we next sought to find whether the circulating aberrant T cells and the infiltrating T cells shared the same clonal rearrangements. We found that, for a given patient, the same clonal TCRγ rearrangement was observed in the last blood sample and in at least one tissue for 8 out of 10 patients and in several biopsies for five patients, despite the delay of several years (maximum 23 years) between the biopsy and the blood sample (Figure 2). In the other cases, the quantity of DNA was insufficient for analysis.

Histopathological and immunophenotypic characteristics of two AITL/L-HES patients

We retrieved all available biopsies and blood samples collected at AITL diagnosis in patients P4 and P16. Histopathological features of lymph node (P4), skin and bone marrow biopsies, immunophenotypic characteristics of circulating CD3-CD4+ T-cells and TCRγ rearrangement studies (P4 and P16) were performed and compared to their own previous samples collected since CD3-CD4+ L-HES diagnosis.

Lymph node

At AITL diagnosis in 2014, patient's P4 lymph node sampled had common histopathological findings with lymph nodes sampled in 2000 and 2011 (Table 2): HEV, effacement of architecture by CD3+CD4+CD5+ lymphoid infiltrates which spared the peripheral cortical sinuses, and presence of a clonal T-cell subset as

demonstrated by TCRγ rearrangement study. But contrary to previous biopsies, neoplastic cells were polymorphic, medium to large sized with clear nucleus and clear and large cytoplasm, and expressed CXCL13 and PD1 (TFH markers) and weakly CD10 (Figure 3), without significant expression of EBER transcripts. As skin and bone marrow histopathological findings were typical in patient P16 (see below), lymph node biopsy was not performed.

Skin

At AITL diagnosis of patient P4, there was a high dermis infiltration by lymphoid cells which were also polymorphic, medium to large sized, with a large and clear cytoplasm, but did not express CD10 and TFH markers (Table 3). At AITL diagnosis in patient P16, there were also important lymphoid infiltrates in all the dermis, and a large involvement of hypodermis composed of lymphoid cells which appeared to be CD3+CD4+, positive for CD10, PD1 and CXCL13 (Table 3, Figure S7A). There were rare large B-cells and no EBV-positive B-cells. In both AITL/L-HES patients, a clonal TCRγ rearrangement was detected in skin biopsy.

Bone marrow

Large CD10 and TFH markers-positive lymphoid infiltrates were found in P4 and P16's bone marrow biopsies. In all other CD3-CD4+ L-HES patients and in both patients P4 and P16 before AITL diagnosis, there was no bone marrow abnormal lymphoid infiltrate. A clonal TCRγ rearrangement was detected in bone marrow biopsy in patient P4 at L-HES diagnosis.

Circulating CD3-CD4+ T-cells

In patient P4, CD3-CD4+ T-cells remained high (between 40-60 G/L since 2011) and did not express CD10 at AITL diagnosis. In patient P16, CD3-CD4+ T-cells subset increased at AITL diagnosis and eighty-five percent of cells were CD10-positive (unfortunately this parameter was not available in previous immunophenotypic analysis) (Figure S7B).

TCRγ rearrangement study

In patient P4, the same TCRγ rearrangement was found in peripheral blood, skin, lymph node and bone marrow biopsies at AITL diagnosis in 2014 and was the same as in previous samples collected since L-HES diagnosis in 2000 (Figure 2, and Figure S8). In patient P16, the same clonal TCRγ rearrangement was detected in skin and peripheral blood at AITL diagnosis in 2010, and was the same as in peripheral blood sample at L-HES diagnosis in 2004 (Figure S7C).

Discussion

To our knowledge, our study demonstrates for the first time that the CD3-CD4+ L-HES is not only a secondary HE to circulating clonal T-cells but a peripheral clonal T-cell lymphoproliferative disorder, characterized by a benign course in the majority of patients despite a nodal and extra-nodal dissemination of clonal T-cells which can persist for many years. We also report two well-defined AITL occurring during CD3-CD4+ L-HES course: the presence of the same clonal T-cells in tumor lesions and in peripheral blood or tissue sampled several years before, confirms the hypothesis of a pre-malignant disease.

Some authors have already considered L-HES as a non-malignant T-cell lymphoproliferative disorder, but this definition was supported by the presence of persistent clonal circulating mature T-cells.[28] Only three reports have previously suggested the dissemination of aberrant CD4+ T-cells into skin or lymph node biopsies in L-HES patients and only based on indirect evidence: absence of CD7 expression, which is also fully absent or partially expressed on circulating T-cells, or a partial lack of anti-CD3 staining in CD4 T-cells.[6,21,29] In our cohort of CD3-CD4+ L-HES patients, which is the largest ever reported so far, we show that lymphoid cells which compose the infiltrates have exactly the same phenotypic characteristics whatever the tissue involved and never express CD10 or TFH markers (CXCL13 and PD1). Since IHC cannot distinguish membranous from cytoplasmic CD3 expression in paraffin section, which is conserved in L-HES,[3] we aimed to demonstrate that CD4+ T-cells in diseased tissues are the same as the circulating cells: analysis of two fresh skin samples by flow cytometry confirmed that CD4+ T-cells predominantly lacked CD3 membranous expression. We can suppose that T-cells are responsible of secondary tissular eosinophilia as suggested by a previous report which showed IL-5 production by skin aberrant T-cells in two cases.[21] On the other hand, the rare cases of isolated tissular eosinophilia (without any detected abnormal lymphoid population) may be explained by blood eosinophils migration into tissues, like digestive tract for patients P11 and P14, or less likely, by very small clonal T-cell amounts in tissue. Furthermore, the same clonal TCRγ rearrangements were found both in peripheral blood and lymph nodes, skin or other extra-nodal tissues in a large majority of available cases. In the other cases, there was no DNA amplification, probably because of an insufficient quantity of DNA and/or because of paraffin-embedding.[27] Blood, nodal and extra-nodal dissemination of these clonal CD3-CD4+ T-cells are in favor of a peripheral clonal T-cell lymphoproliferative disorder.

The clonal CD3-CD4+ T-cells can infiltrate various tissues and they can persist for several years (up to 23) in the same patient without developing a T-cell lymphoma: indeed, like indolent B-cell lymphoproliferative disorders such as monoclonal B-cell lymphocytosis or mucosa-associated lymphoid tissue lymphoma, CD3-CD4+ L-HES follows a benign course in the majority of patients. Corticosteroids are always effective on symptoms and on eosinophil count. Corticosteroids alone or in association with interferon-α therapy are also able to induce a decrease of CD3-CD4+ circulating T-cell count,[2,13,22,30] like patient P10 in this study, which further highlights the indolent behavior of this disease. This observation is in accordance with the good health status of CD3-CD4+ L-HES patients despite a course over several years.

Altogether these data favor the hypothesis that CD3-CD4+ L-HES is a benign peripheral clonal T-cell lymphoproliferative disorder which should not be misdiagnosed (and treated) as an aggressive malignant disease.

Nevertheless, some previous studies reported PCTLs during L-HES course, including one AITL, and we describe two AITL cases in our patients: some objective and easy-to-use criteria are needed to distinguish L-HES from T-cell lymphoma, and especially AITL which shares many clinical manifestations (skin lesions, lymphadenopathy, various peripheral manifestations such as joint or lung involvement…), biological (HE, circulating CD3-CD4+ T-cells, …) and histopathological characteristics.[31,32] Indeed, circulating aberrant T-cells in AITL seem to have the same cytological characteristics as CD3-CD4+ T-cells in L-HES.[33] In skin, CD3-CD4+ L-HES and AITL are characterized by similar infiltrates in the superficial dermis, rarely in the deep dermis, without epidermotropism.[34,35] In lymph nodes, AITL is also characterized by partial effacement of the architecture, often with perinodal infiltration but peripheral cortical sinuses are spared. There is a proliferation of arborizing HEV and an increased CD23+ follicular dendritic cell meshwork. The infiltrate is composed of atypical lymphoid cells, eosinophils and plasma cells.[34] Abnormal T-cells appear to be CD3+CD4+CD5+ in both entities but these cells express TFH markers (ie CXCL13, PD1) and CD10 specifically in AITL,[34] and in none of our CD3-CD4+ L-HES patients. Coherently, CD10 and TFH markers were detected in biopsies at AITL diagnosis in both AITL/L-HES patients P4 and P16. Reanalysis of patient P4's lymph nodes in 2000 (CD3-CD4+ L-HES diagnosis) and in 2011 (follow-up) did not find these markers and confirmed their interest for AITL diagnosis during CD3-CD4+ L-HES course. The TFH markers were not detected in P4's skin biopsy whereas both morphologic features were in favor AITL in skin and TFH markers were detected in lymph node in the same patient. The presence of EBV-positive B-cells, another characteristic of AITL, was never found in both CD3-CD4+ L-HES patients, and in P4's lymph nodes at AITL diagnosis.

Increased expression of CD2 and/or CD5, loss or diminished expression of CD7 are usually found in CD3-CD4+ T-cells in both L-HES and AITL.[16–18,36–38] But circulating CD3-CD4+ T-cells never express CD10 in CD3-CD4+ L-HES, except in patient P16 at AITL diagnosis.

In previously reported lymphoma cases during L-HES course, the diagnosis of lymphoma relied on the presence of enlarged lymph nodes, progression of skin lesions to infiltrative nodules, presence of dermal and nodal infiltration by atypical lymphoid cells, sometimes with clonal TCR rearrangement, nodular infiltration by T-cells with the same phenotype as circulating T-cells and/or a recent increase in blood CD3-CD4+ T-cells.[3,6,19–21] Our study shows that all these conditions can also be observed in L-HES without lymphoma. Clinicians and pathologists managing these patients should be aware that a T-cell lymphoma, requiring aggressive chemotherapy, should not be diagnosed in a CD3-CD4+ L-HES patient exclusively on the basis of an infiltrative clonal CD4+ T-cells disease or architectural modification of lymph nodes. Recently, some other T-cell and NK-cell lymphoproliferative disorders mimicking peripheral T-cell and NK-cell lymphomas were identified by the lymphoma workshop of the European Association for Haematology/Society for Hematopathology.[39] We propose to define CD3-CD4+ L-HES as a "peripheral

indolent clonal T-cell lymphoproliferative disorder" to highlight the clear distinction between indolent disease and aggressive malignant lymphoma. Many single or overlapping mutations have been recently reported in AITL. *TET2, DNMT3A* and *IDH2* mutations were searched by NGS using the Ion Torrent Proton instrument in samples of our both AITL patients: only one TET2 A1876E mutation was found in patient P16's skin lesions, in 9% of extracted DNA. There was no detected mutation in patient P4's skin, bone marrow and lymph node biopsies performed at AITL diagnosis (data not shown). Larger studies are needed to assess the significance of such mutations in CD3-CD4+ L-HES-related AITL.

In conclusion, our study shows that CD3-CD4+ L-HES can be considered as an indolent T-cell lymphoproliferative disorder with blood, nodal and extra-nodal involvement. CD3-CD4+ T-cells can persist over years without transformation in lymphoma, as demonstrated by repeated biopsies showing persistent clonal T-cells infiltrates. We also reported the two first well-documented cases of AITL, according to the 2008 WHO classification. We have both to increase our series of patients with long-term follow-up and to understand the molecular mechanisms underlying the development of this disorder, to evaluate the risk of AITL in L-HES and the biological link between these two entities. Compared to L-HES, clonal lymphoid cells at AITL diagnosis had larger and clearer nuclei, more abundant cytoplasm. CD10 and TFH markers, the most specific histopathological characteristics of AITL, were only observed in both AITL/L-HES cases. Cell morphological changes and absence of TFH markers and CD10-positive T-cells are easy-to-use negative arguments in favor of the indolent disease.

Authorship and disclosures

GL, M-CC, CR, NG, LP, DL, CP and J-EK designed the study and analyzed the data.

GL, HA, MA-A, DS-S, JS, GS, KG, LT, CL, CM-H, FM, OL, FA, AT, P-YH, LP, DL, and J-EK observed the patients and collected the data.

GL, CR, JT, FD and ML performed and interpreted flow cytometry analysis.

M-CC performed histopathological analysis

NG performed and interpreted TCRγ rearrangement analysis

CR, SP and SG designed and interpreted *TET2, DNMT3A* and *IDH2* sequencing.

GS, LT, FM, MC, CR-L, and LP interpreted data and critically reviewed the manuscript.

GL, M-CC, CR, NG, DL, CP, J-EK wrote the paper

All authors approved the final manuscript.

The authors declare no competing financial interests.

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Table 1. Clinical characteristics, available biopsies and blood samples in the CD3-CD4+ L-HES patients

Patients	Age at HES diagnosis	Sex	Date of first blood HE	Date of first HES symptoms	Organs involvement	Available biopsies			Last available blood sample	Treatments received during HES follow-up	Disease duration (years) [a]	Status at the end of follow-up
						Skin	Lymph nodes	Other extra-nodal sites				
P1	42	M	1990	1990	Skin, lymph nodes, tonsils, lacrymal glands, parotid, subcutaneous nodules	2011, 2013	2013	Lacrymal gland (1990)	2013	CS, CSA, MTX, Phototherapy, peg-IFNα	24	Alive
P2	68	M	2006	2006	Skin	2008, 2010, 2013			2013	CS	7	Alive
P3	51	F	1996	2000	Skin, lymph nodes	2006, 2007, 2008, 2009, 2010	2006, 2007		2011	HU, IM, multiple lines chemotherapy, aSCT	17	Died (infectious adverse event after aSCT)
P4	18	M	1999	1999	Skin, lymph nodes, episodic angioedema, joints, spleen	2002, 2011 2014 (AITL)	2000, 2011 2014 (AITL)	BM (2000: L-HES ; 2014: AITL)	2014	CS	15	Died (AITL, 2014)
P5	31	F	2005	2007	Skin, joints, digestive tract	2009		Synovium (2007), Digestive tract (2011)	2014	CS	8	Alive
P6	43	F	2010	2010	Skin, lymph nodes, joints	2010, 2012			2012	CS, IFN α	4	Alive
P7	57	F	2005	2005	Skin	2005			2013	no treatment	9	Alive
P8	18	F	1995	1995	Skin, lymph nodes, joints	2002, 2005, 2009, 2011, 2014			2014	CS, IFNα, IM, MEPO	19	Alive
P9	38	F	1996	1996	Skin, central nervous system, arterial aneurysms	2010			2014	CS, IM, MEPO	18	Alive
P10	61	F	1987	2009	Lymph nodes, digestive tract		2011	Digestive tract (2009)	2013	CS, CSA	27	Alive
P11	43	F	1991	1991	Skin, joints, digestive tract, bronchus			Digestive tract (2004)	2013	CS, IM, MEPO	23	Alive
P12	16	M	2007	2007	Skin, lymph nodes, episodic angioedema, spleen				2013	CS	7	Alive
P13	75	F	2006		No symptom				2014	no treatment	8	Alive
P14	50	F	1993	2005	Skin, digestive tract			Digestive tract (2005, 2012)	2013	CS, MTX, HU, IFNα, MEPO	21	Alive
P15	36	M	2008	2009	Skin, lymph nodes, central nervous system				2010	CS, MTX, CYC, ALEM	4	Alive
P16	52	M	2002	2002	Skin, episodic angioedema,	2010 (AITL)		BM (2004: L-HES ;	2010	CS, IFNα	8	Died

joints	2010: AITL)	(AITL, 2010)

[a] Disease duration is defined as the interval between the first HES symptom and/or the first HE observed on a blood numeration, and the last visit or AITL diagnosis.

L-HES, lymphoid variant of hypereosinophilic syndrome; CS, corticosteroids; CSA, ciclosporin A; IFNα, interferon α; MEPO, mepolizumab; MTX, methotrexate; HU, hydroxycarbamide; IM, imatinib; aSCT, allogenic stem-cells transplantation; AITL, angioimmunoblastic T-cell lymphoma; CYC, cyclophosphamide; ALEM, alemtuzumab.

Table 2. Lymph nodes histopathology in CD3-CD4+ L-HES and in one AITL occurred in a CD3-CD4+ L-HES patient (P4)

Patients	Diagnosis and available samples		Morphology of atypical T-cells			Pattern of T-cells infiltration		Increased and/or disorganized FDC CD23+ meshwork[2]	HEV[2]	Eos.[2]	Plasm.[2]	EBV+ B-cells[2]	Immunophenotypic profile of T-cells[2]				Clonal TCRγ rearr.[3]
			Size	Nucleus	Cytopl.[1]	Level of effacement of architecture by infiltrates	Respect of peripheral cortical sinuses[2]						CD3+ CD4+ CD5+	CD10	PD1	CXCL13	
P1	L-HES	2013	s/m	irregular, dense	+	n.a (needle biopsy)	+	++	+	++	+/-	0	+	0	0	0	+
P3	L-HES	2006	s/m	irregular, dense	++	interfollicular	+	+/-	+/-	+++ (IF)	+	0	+	0	0	0	n.a
	L-HES	2007	s/m	irregular, dense		90%	+	+/-	+	+	+	0	+	0	0	0	+
P4	L-HES	2000	s/m	irregular, dense	++	80%	+	+	+	+	+	0	+	0	0	0	+
	L-HES	2011	s/m	irregular, dense		80%	+	+	+	+	+	0	+	0	0	0	+
	AITL	**2014**	**m/l**	**large, clear**	**+++**	**100%**	**+**	**+**	**+**	**+/-**	**+**	**-**	**+**	**+**	**+**	**+**	**+**
P10	L-HES	2009	s/m	irregular, dense	++	interfollicular	+	+	+	+++ (IF)	+	0	+	0	0	0	+

[1] *+ scarce cytoplasm, ++ quiet abundant cytoplasm, +++ abundant cytoplasm*

[2] *0: absent condition or absence of cells; +/-: moderate infiltrates or rare cells; +: present condition or present cells; ++ or +++: increased FDC CD23+ mesh work, high density of concerned cells*

[3] *clonal TCRγ rearrangement is indicated as present ("+"). In patient P3, there was no amplification probably due to the DNA degradation in paraffin-embedding and was considered as not available ("n.a").*

Cytopl, cytoplasm; FDC, follicular dendritic cells ; Eos, eosinophils; Plasm, plasma cells; TCRγ rearr, T-Cell Receptor gamma rearrangement; L-HES, lymphoid variant of hypereosinophilic syndrome; n.a, not available; s/m, small-to-medium size; IF, interfollicular infiltrates; AITL / L-HES, angioimmunoblastic T-cell lymphoma occurred during L-HES course

Table 3. Skin histopathology in CD3-CD4+ L-HES and in two AITL occurred in CD3-CD4+ L-HES patients (P4 and P16)

Patients	Diagnosis and number of available samples[1]	Morphology of atypical T-cells			Pattern of T-cells infiltration								Immunophenotypic profile of T-cells[3]				Clonal TCRγ rearr[4]
		Size	Nucleus	Cytopl.[2]	Epid.[3]	Dermis					Hypod.[3]	Eos.[3]	Plasm.[3]	CD3+ CD4+ CD5+	CD10	PD1	CXCL13	
						→EpiD[3]	S/D	N/D	PV[3]	PA[3]								
P1	L-HES (2)	s/m	irregular, dense	+	0	0	S/D	N	+	+	0	+	+/-	+	0	0	0	+
P2	L-HES (4)	s/m	irregular, dense	+	0	0	S/D	N	+	+	+	+	+/-	+	0	0	0	+
P3	L-HES (5)	s/m	irregular, dense	+	0	0	S	N	+	0	0	+	+/-	+	0	0	0	+
P4	L-HES (2)	s/m	irregular, dense	++	0	0	S/D	N/D	+	0	0	+/-	+/-	+	0	0	0	+
	AITL (1)	**m/l**	**large, clear**	**+++**	**0**	**0**	**S/D**	**D**	**+**	**+**	**n.a**	**+**	**+/-**	**+**	**0**	**0**	**0**	**+**
P5	L-HES (1)	s/m	irregular, dense	+	0	0	S	N	+	0	0	+	0	+	0	0	0	n.a
P6	L-HES (2)	s/m	irregular, dense	+	0	0	S/D	N	+	+	++	+	+/-	+	0	0	0	n.a
P7	L-HES (1)	s/m	irregular, dense	+	0	0	S	N	+	0	0	+	0	+	0	0	0	+
P8	L-HES (4)	s/m	irregular, dense	+	0	0	S	N	+	0	0	+	+/-	+	0	0	0	-
P9	L-HES (1)	s/m	irregular, dense	+	0	0	S	N	+	0	0	0	0	+	0	0	0	+
P16	**AITL (1)**	**s/m**	**irregular, dense**	**++**	**0**	**0**	**S/D**	**N**	**+**	**+**	**++**	**+**	**+/-**	**+**	**+**	**+**	**+**	**+**

[1] Skin lesions in L-HES patients were pruritic papulo-nodular inpatients P1 and P2, pruritic papular lesions in patient P3, eczema-like lesions in patients P4, P5, P8, maculo-papular lesions in patient P6, isolated pruritus in patients P7 and P9. Numbers in parentheses are the number of available biopsies for each patient: in all cases, the same characteristics were observed in each sample, with the exception of the intensity of the lymphoid and eosinophils infiltrates which could change from one biopsy to another.

[2] + scarce cytoplasm, ++ quiet abundant cytoplasm, +++ abundant cytoplasm

[3] 0: absent condition or absence of cells; +/-: moderate infiltrates or rare cells; +: present condition or present cells; ++ or +++: high density of concerned cells; n.a: not available

[4] clonal TCRγ rearrangement is indicated as present ("+") if it was detected in at least one biopsy. In patients P5 and P6, there was not enough extracted DNA to make the analysis, and was considered as not available ("n.a"). In patient P7, a clonal TCRγ rearrangement was detected on fresh biopsy in 2005, but not on the same paraffin-embedded sample which was retrieved and retested in 2014, despite sufficient quantity of DNA.

Cytopl, cytoplasm; EpiD, epidermis; →EpiD, epidermotropism ; S/D, superficial and/or deep dermis involvement; N/D, nodular and diffused infiltrates; PV, perivascular; PA, periadnexal; Hypod., hypodermis; Eos, eosinophils; Plasm, plasma cells; TCRγ rearr, T-Cell Receptor γ rearrangement; L-HES, lymphoid variant of hypereosinophilic syndrome; s/m, small-to-medium size; n.a, not available; AITL / L-HES, angioimmunoblastic T-cell lymphoma occurred during L-HES course.

Table 4. Other extra-nodal tissues histopathology in CD3-CD4+ L-HES

Patients	Diagnosis	Available samples[1]	Lymphoid infiltrates[2]	Morphology of atypical T-cells			Pattern of T-cells infiltration	Eos.[3]	Plasm.[3]	EBV+ B-cells[3]	Immunophenotypic profile of T-cells[3]				Clonal TCRγ rearr.
				Size	Nucleus	Cytopl.[2]					CD3+ CD4+ CD5+	CD10	PD1	CXCL13	
P1	L-HES 1990	Lacrymal gland	+++	s/m	irregular, dense	+	Dense lymphoid infiltrates, partial effacement of lacrymal gland.	+	+	0	+	0	0	0	+
P5	L-HES 2007	Synovium	+++	s/m	irregular, dense	++	Dense lymphoid infiltrates, thickened synovium.	+	+	0	+	0	0	0	+
	L-HES 2011	Digestive tract	0					0							
P10	L-HES 2009	Colon	+++	s/m	irregular, dense	+	Dense lymphoid infiltrates, partial effacement of colon glands.	+	+	0	+	0	0	0	+
P11	L-HES 2004	Digestive tract	0					+							
P14	L-HES 2005	Digestive tract	0					+							
	L-HES 2012	Digestive tract	0					0							

[1] Patients P5, P10, P11, P14 had multiple digestive biopsies (stomach, duodenum, colon). Patient P10 had eosinophils and lymphoid infiltrates only in colon, there was no eosinophils or lymphoid infiltrates in patient P5 who had only a suspicion of malabsorption without clinical manifestation, there was only eosinophils infiltrates in P11 and P14's biopsies (only in 2005 for P14, not on the second one which was performed under corticosteroids)

[2] 0: absent condition or absence of cells; +/-: moderate infiltrates or rare cells; +: present condition or present cells; ++ or +++: high density of concerned cells

[3] + scarce cytoplasm, ++ quiet abundant cytoplasm

Cytopl, cytoplasm; Eos, eosinophils; Plasm, plasma cells; TCRγrearr, T-Cell Receptor γrearrangement; L-HES, lymphoid variant of hypereosinophilic syndrome; s/m, small-to-medium size.

Table 5. Bone marrow histopathology in two CD3-CD4+ L-HES patients who developed AITL

Patients	Diagnosis and available samples	Lymphoid infiltrates	Morphology of atypical T-cells			Pattern of T-cells infiltration	Eos.[2]	Plasm.[2]	EBV+ B-cells[2]	Immunophenotypic profile of T-cells[2]				Clonal TCRγ rearr.
			Size	Nucleus	Cytopl.[1]					CD3+ CD4+ CD5+	CD10	PD1	CXCL13	
P4	L-HES (2000)	0					+++							n.a
	AITL (2014)	**++**	**m/l**	**Large, clear**	**+++**	**N/D, perivascular and paratrabecular**	**+**	**+**	**0**	**+**	**+**	**+**	**+**	**+**
P16	L-HES (2004)	0					+++							n.a
	AITL (2010)	**+++**	**s/m**	**irregular, dense**	**+**	**N/D, perivascular and paratrabecular**	**++**	**+/-**	**0**	**+**	**+**	**+**	**+**	**+**

[1] *+ scarce cytoplasm, ++ quiet abundant cytoplasm, +++ abundant cytoplasm*

[2] *0: absent condition or absence of cells; +/-: moderate infiltrates or rare cells; +: present condition or present cells; ++ or +++: high density of concerned cells*

Cytopl, cytoplasm; Eos, eosinophils; Plasm, plasma cells; TCR γrearr, T-Cell Receptor γrearrangement; L-HES, lymphoid variant of hypereosinophilic syndrome; m/l, medium-to-large sized; N/D: nodular and diffused infiltrates; s/m, small-to-medium size; n.a: not available

Figures legends

Figure 1. Skin histopathology in CD3-CD4+ L-HES.

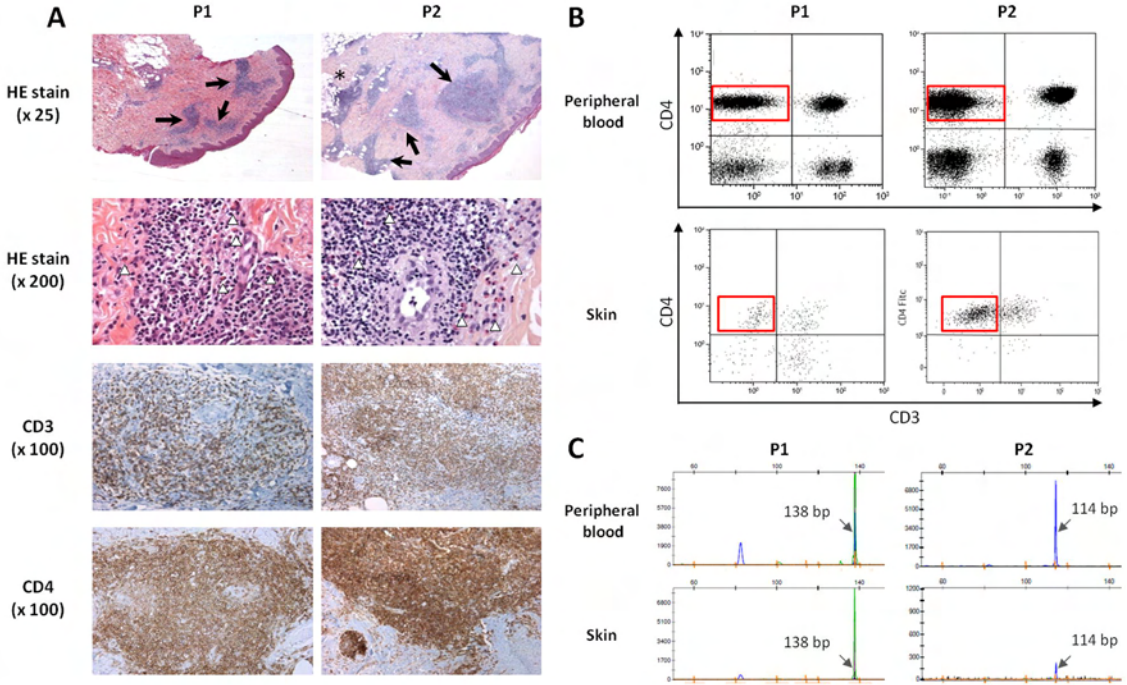
Hematoxylin and Eosin (HE) staining of these two representative skin biopsies (patients P1 and P2) reveal dense nodular, periadnexal and perivascular infiltrates (black arrows), a hypodermis infiltrate in P2 (*). Lymphoid cells are small to medium-sized, with irregular nucleus and scarce cytoplasm. Numerous eosinophils are also observed (Δ). Cells appear to be CD3+CD4+ (A), there are no CD10, CXCL13 and PD1-positive cells (not shown). Flow cytometry confirms the presence of CD3-CD4+ T-cells in skin lesions (B). The same clonal TCRγ rearrangement is detected in skin and blood samples (C).

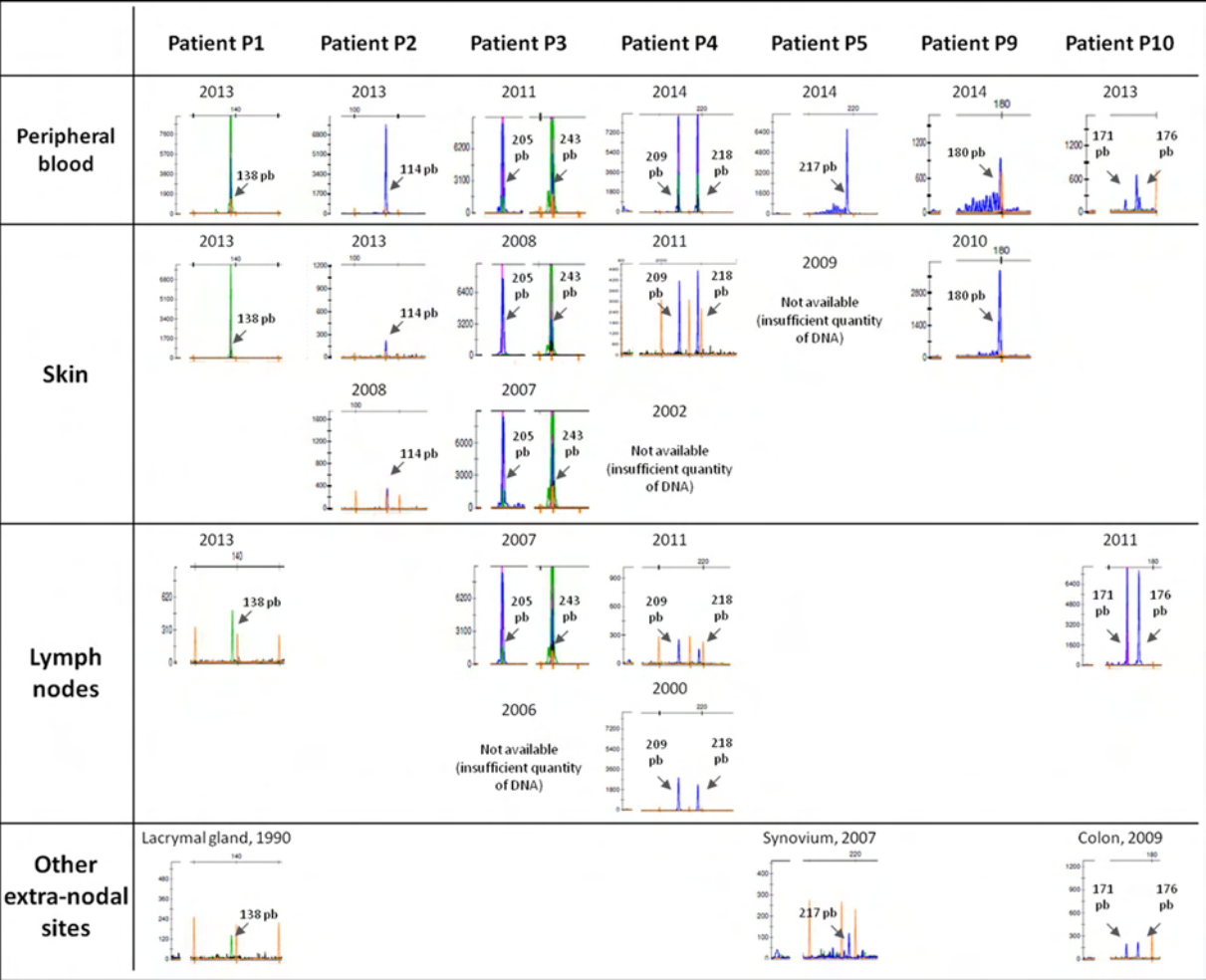
Figure 2. Persistence of clonal T-cells in peripheral blood and various tissues CD3-CD4+ L-HES patients.

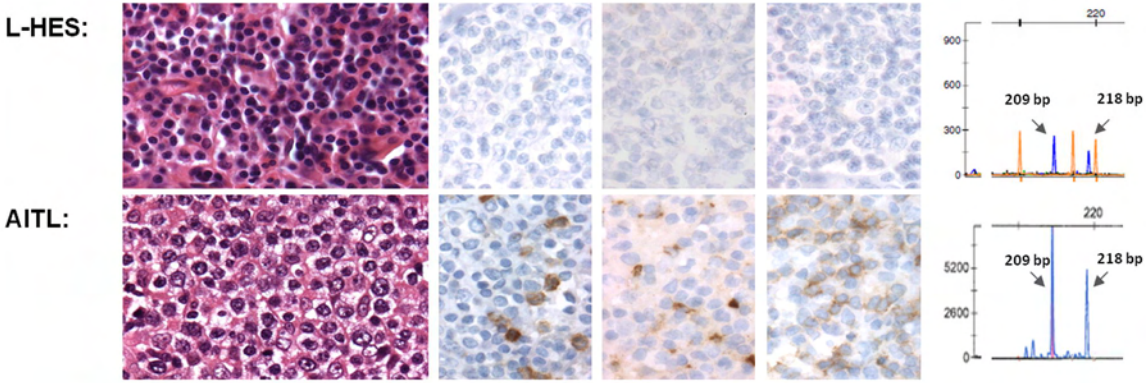
Comparison of TCR rearrangement (γ1-10 and γ9-11 family genes) between the most recent peripheral blood sample and various tissues biopsies in seven representative CD3-CD4+ L-HES patients. Note the spatial dissemination of the clonal T-cell in various sites, including peripheral blood, and its persistence over years in all patients. Patient P4's samples presented here have been collected during L-HES course, before AITL diagnosis.

Figure 3. Comparison of cytological characteristics of lymphoid cells in CD3-CD4+ L-HES and at AITL diagnosis in patient P4.

In context of CD3-CD4+ L-HES (lymph node biopsy performed during follow-up in 2011), lymphoid cells were monomorphic, small to medium sized, with irregular nucleus and scarce cytoplasm (*HE,* Hematoxylin and Eosin staining), and did not express CD10, CXCL13 or PD1. At AITL diagnosis in 2014, neoplastic cells were polymorphic, medium to large sized with clear nucleus and clear and large cytoplasm, and some C10, CXCL13 and/or PD1-positive cells are found. In both cases, the same clonal TCRγ rearrangement was detected.


A
P1
P2
HE stain (x 25)
HE stain (x 200)
CD3 (x 100)
CD4 (x 100)
B
P1
P2
Peripheral blood
Skin
CD4
CD3
CD4 Fitc
C
P1
P2
Peripheral blood
Skin
138 bp
114 bp
138 bp
114 bp


Patient P1
Patient P2
Patient P3
Patient P4
Patient P5
Patient P9
Patient P10
Peripheral blood
Skin
Lymph nodes
Other extra-nodal sites
2013
138 pb
2013
114 pb
2011
205 pb
243 pb
2014
209 pb
218 pb
2014
217 pb
2014
180 pb
2013
171 pb
176 pb
2013
138 pb
2013
114 pb
2008
205 pb
243 pb
2011
209 pb
218 pb
2009
Not available (insufficient quantity of DNA)
2010
180 pb
2008
114 pb
2007
205 pb
243 pb
2002
Not available (insufficient quantity of DNA)
2013
138 pb
2007
205 pb
243 pb
2011
209 pb
218 pb
2011
171 pb
176 pb
2006
Not available (insufficient quantity of DNA)
2000
209 pb
218 pb
Lacrymal gland, 1990
138 pb
Synovium, 2007
217 pb
Colon, 2009
171 pb
176 pb


L-HES:
AITL:
220
900
600
300
0
209 bp
218 bp
220
5200
2600
0
209 bp
218 bp
HE stain (x 40)
CD10 (x 40)
CXCL13 (x 40)
PD1 (x 40)

Supplemental information

CD3-CD4+ lymphoid variant of hypereosinophilic syndrome: nodal and extranodal histopathological and immunophenotypic features of a peripheral indolent clonal T-cell lymphoproliferative disorder.

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* These authors contributed equally to this work, ** These authors contributed equally to this work

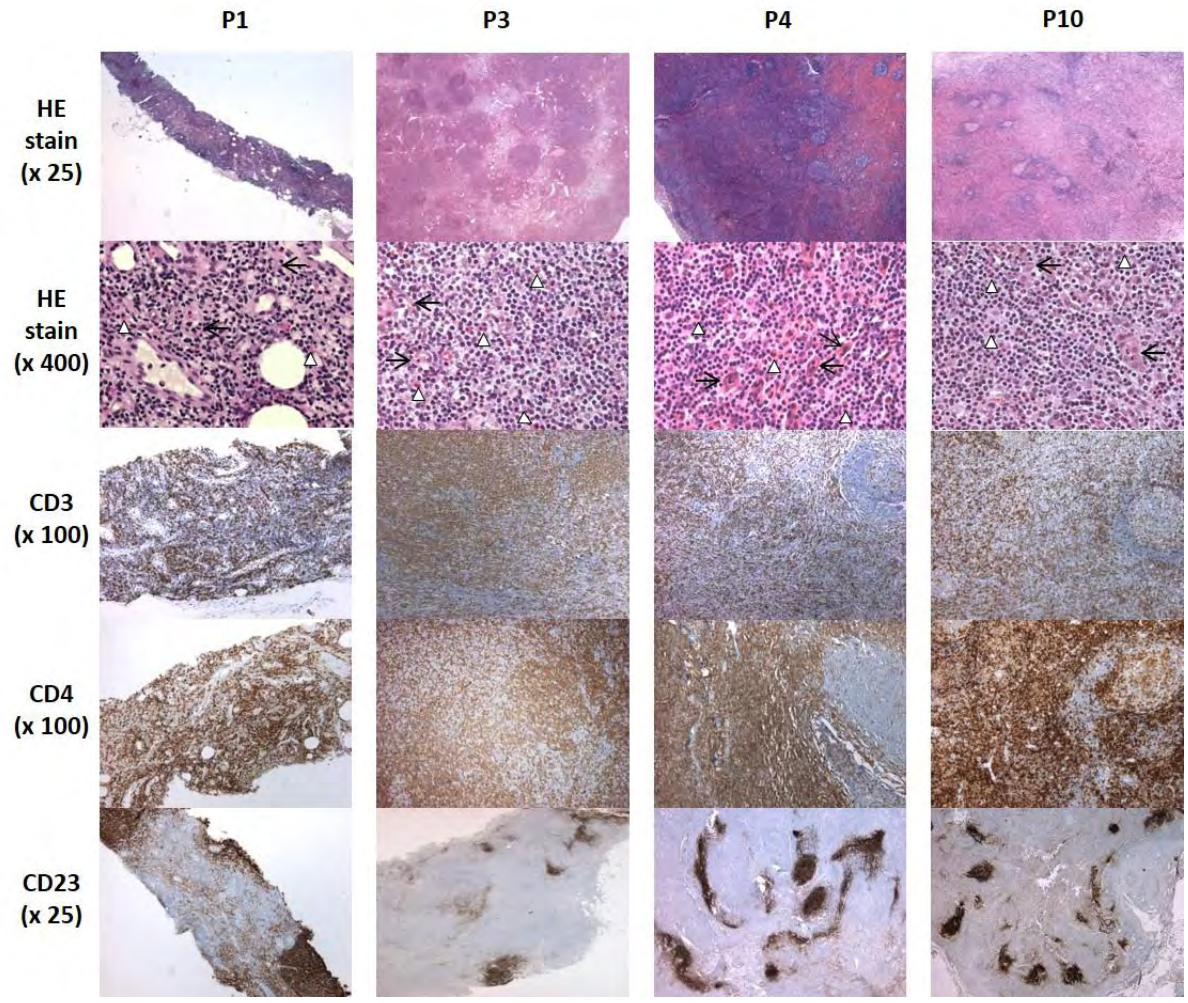


Figure S1. Lymph nodes histopathology in CD3-CD4+ L-HES

Histopathological characteristics of lymph nodes (LN) in 4 CD3-CD4+ L-HES patients. Hematoxylin and Eosin (HE) staining revealed a slight (ex: patient P10) to dense lymphoid infiltration responsible of an effacement of LN architecture (patients P3 and P4). LN architecture is not appreciable on LN needle biopsy of patient P1. In all cases, lymphoid infiltrates spare the peripheral cortical sinuses (not appreciable in patient P1) and cases are composed of CD3+CD4+ T-cells, without any CD10, CXCL13 or PD1 positive T-cells (not shown). The other characteristics are the presence of numerous eosinophils (Δ), rare plasma cells, high endothelial veinules (black arrows), and increased of CD23+ follicular dendritic cells meshwork.

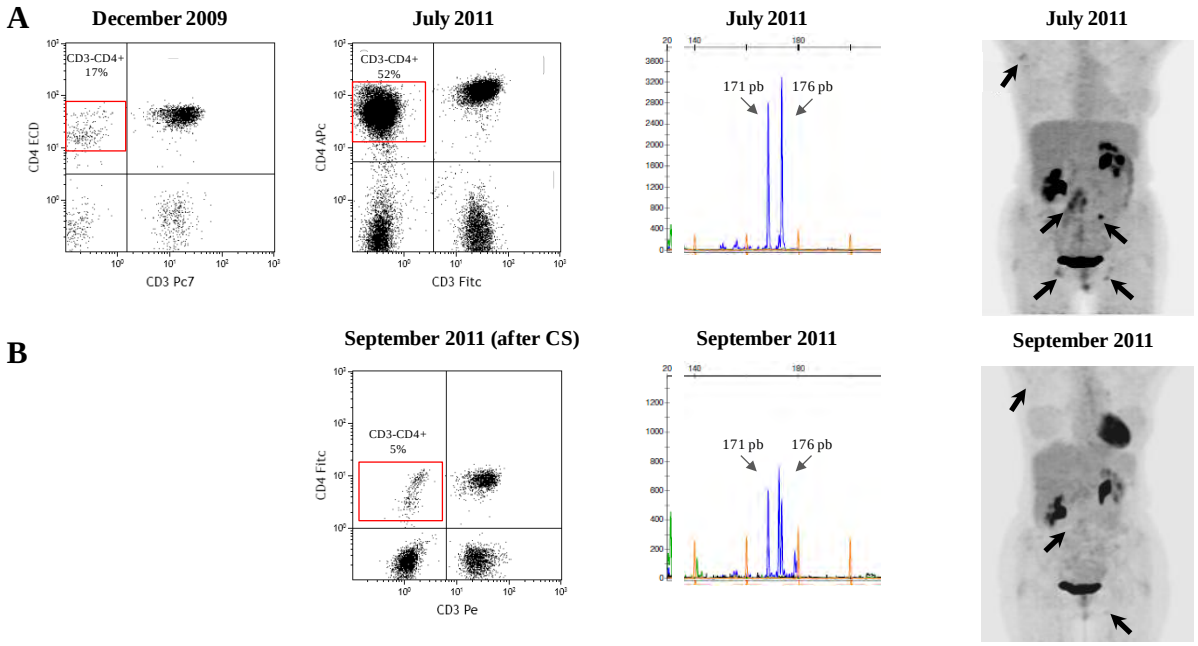
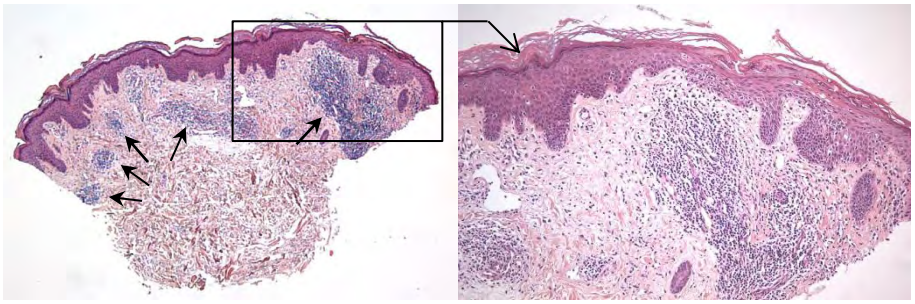


Figure S2. Favorable evolution of lymph node involvement in a CD3-CD4+ L-HES patient (P10).

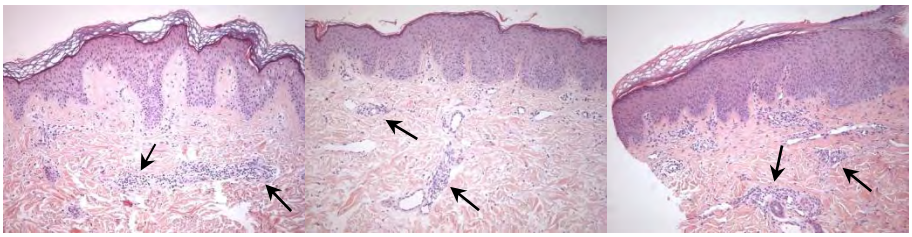
CD3-CD4+ L-HES diagnosis made in 2009 in patient P10 was based on a long-history of blood HE (23 years), an eosinophilic colitis (atypical CD4+ lymphoid infiltrates were observed a posteriori), circulating CD3-CD4+/low T-cells and a clonal TCRγ rearrangement. In July 2011, digestive symptoms worsened, eosinophils count increased to 23 G/L and she developed multiple adenopathies. CD3-CD4+ T-cell subsets increased from 17% to 52% of total lymphocytes, TCRγ rearrangement study showed the same circulating T-cell clone as in the lymph node (see Fig 2) and 18-Fluoro-deoxyglucose positron emission tomography (18-FDG-PET) showed multiple adenopathies (arrows) (A). Histopathological examination of a coeliomesenteric lymph node did not conclude in a lymphoma (Figure S1) and corticosteroids were started at 0.5 mg/kg/day: 2 months later, the eosinophil count became normal, digestive symptoms disappeared, the circulating CD3-CD4+ T-cell subset decreased, clonal TCRγ rearrangement persisted and 18-FDG-PET dramatically improved (B).

A: 2002



HE stain x25 HE stain x100

B: 2005, 2009, 2011



HE stain x25 HE stain x25 HE stain x25

Figure S3. Persistence of skin lymphoid infiltrates in a CD3-CD4+ L-HES patient (Ex: patient P8)

At diagnosis in 2002 (A), skin biopsy found dense nodular and perivascular lymphoid infiltrates (arrows) composed of CD2+CD3+CD4+CD5+ T-cell infiltrates (immunohistochemistry not shown), which decreased but persisted over years (B) under corticosteroids alone (since 2002), and then after corticosteroids and mepolizumab (since 2006). Coherently, the first T-cell immunophenotype performed in 2005 showed a CD3-CD4+ circulating subset which decreased from 21% (0,71 G/L) to 6,9% (0,11 G/L) in 2012.

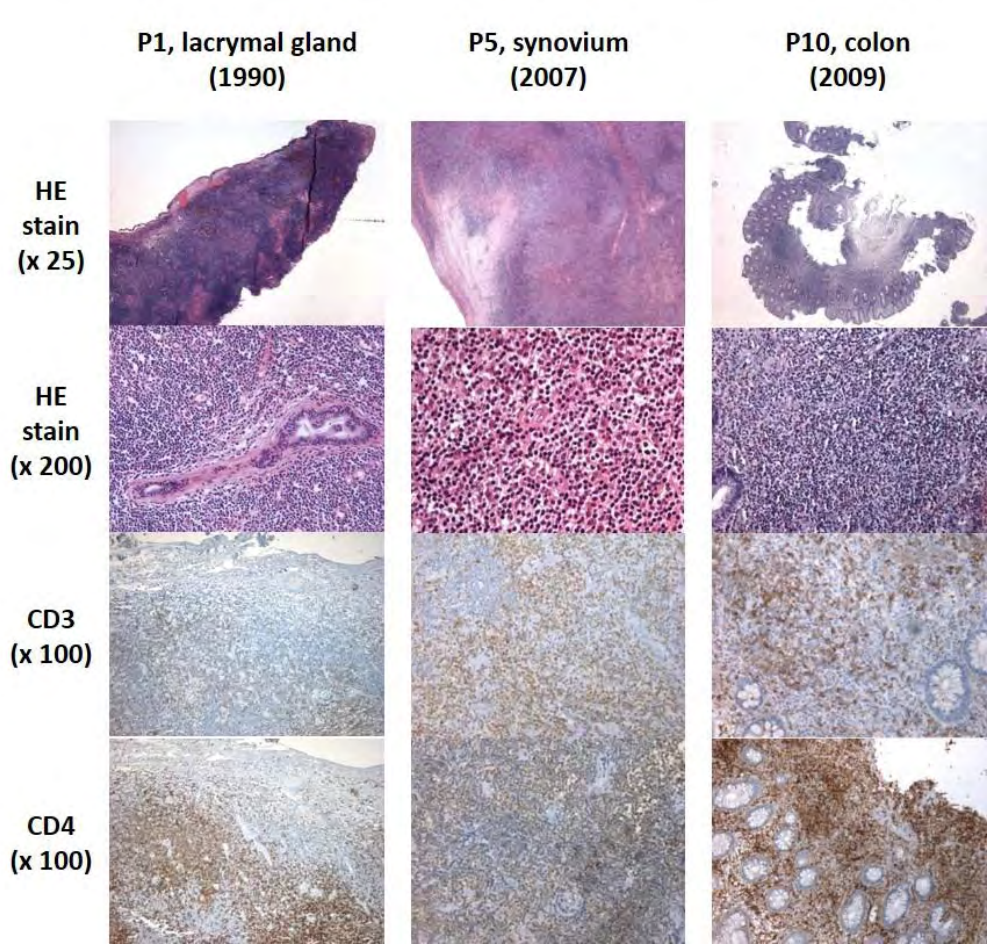


Figure S4. Other extra-nodal tissue histopathology in CD3-CD4+ L-HES

Patient P1 had a refractory generalized eczema and “Kimura like” disease with parotid gland enlargement (biopsy demonstrated an infiltration by eosinophils, but was not available for reanalysis), enlarged cervical lymph nodes, subcutaneous swelling, but also lacrymal gland enlargement. The lacrymal glands were highly modified by dense inflammatory infiltrates composed of lymphoid cells and eosinophils. The same eosinophil and lymphoid infiltrates were found in a synovial biopsy of patient P5 who presented a bilateral teno-synovitis of the wrists and intercarpal joints in 2007, and in a colon biopsy of patient P10 who received the diagnosis of eosinophilic colitis in 2009. In all cases, immunohistochemical staining revealed the presence of CD3+CD4+ T-cells without any CD10, CXCL13 or PD1 positive T-cells (not shown). A clonal TCRγ rearrangement was detected in all three tissues and still detected in peripheral blood of all three patients in 2013-2014, 23, 7 and 5 years after these biopsies, respectively. All three patients remain in good health status under low-dose corticosteroids (CS) alone (P5 and P10), or under CS and interferon-alpha (P1).

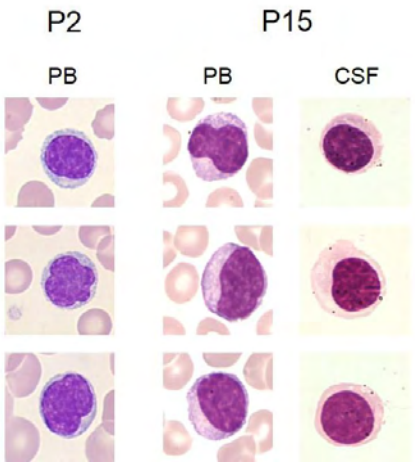


Figure S5. Morphology of circulating T-cells of two representative patients, and in cerebrospinal fluid in one patient

As shown here for patients P2 and P15, aberrant circulating T-cells (PB, peripheral blood) were small to medium-sized, with regular nuclei with condensed and sometimes clumped chromatin, or irregular, indented nuclei, with a moderately abundant cytoplasm (such cytological abnormalities was observed in patients with more than 20 % of aberrant T-cells among total lymphocytes, and were confirmed by comparing CD3-CD4+ versus CD3+CD4+ sorted T-cells in 3 patients). In patient P15 who presented a neuro-meningeal involvement, atypical lymphocytes were found both in blood (left) and cerebrospinal fluid (CSF, right).

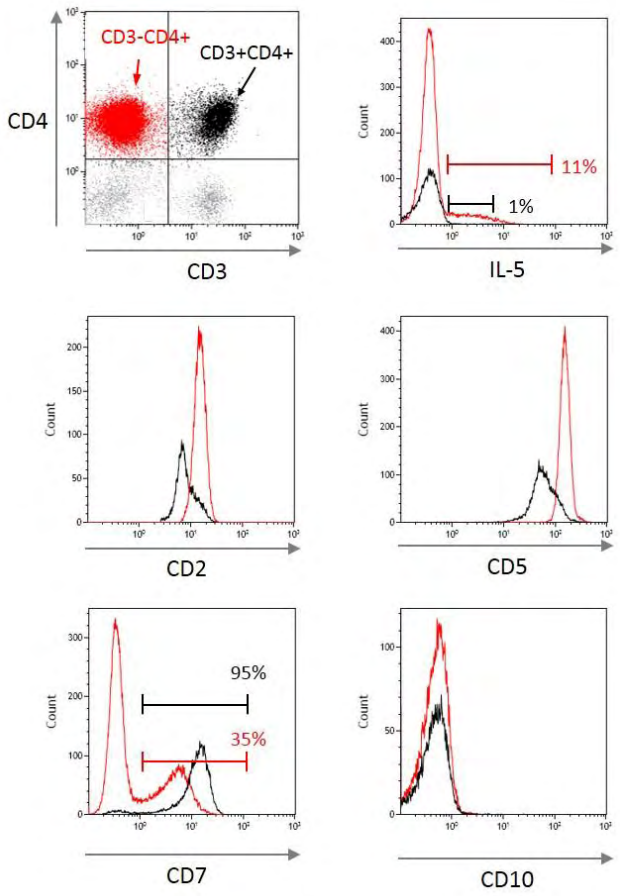


Figure S6. Typical immunophenotyping characteristics of CD3-CD4+ circulating T- cells in L-HES (Ex: Patient P14).

In CD3-CD4+ L-HES, aberrant T-cells produce IL-5 which promotes survival and proliferation of eosinophils (NB: intracellular IL-5 production is detected in permeabilized cells after stimulation by phorbol 12-myristate 13-acetate combined with ionophore, PMA-iono). CD3-CD4+ T-cells (red), compared to CD3+CD4+ T-cells (black), share the same surface markers expression with AITL (CD2+/hi, CD5+/hi, CD7-/low). But unlike AITL, CD3-CD4+ T-cells were always CD10-negative in our L-HES patients.

Supplemental Table S1. Immunophenotyping characteristics of circulating CD3-CD4+ T-cells in L-HES

Patients	Percentage of CD3-CD4+ T-cells among total lymphocytes during follow-up (min-max)	CD3-CD4+ T-cells characteristics at last sample					
		% of total lymphocytes	CD2	CD5	CD7*	CD10	TCRγ rearr
P1	24-78%	46	+	hi	n.a	-	+
P2	39-52%	50	hi	hi	low (16%)	-	+
P3	81-90%	81	+	hi	-	-	+
P4	84-98%	98	+	hi	-	-	+
P5	7-25%	7	hi	hi	low (30%)	-	+
P6	11-17%	11	n.a	n.a	low (6%)	n.a	+
P7	12%-31%	31	hi	hi	-	-	+
P8	7-22%	7	hi	hi	low (28%)	-	+
P9	3.5-7%	3.5	hi	hi	low (10%)	-	+
P10	3-45%	4	+	low	low (45%)	-	+
P11	6-18%	18	+	hi	low (40%)	-	+
P12	2,5-6%	3	hi	hi	low (22%)	-	-
P13	5-7%	6	hi	low	low (16%)	-	+
P14	34-60%	52	hi	hi	low (35%)	-	+
P15	65-79%	75	hi	hi	-	-	+

Abbreviations: TCRγ rearr, clonal T-Cell Receptor γ rearrangement; +, presence; -, absence; hi, high expression; lo, low expression; n.a, not available.

*Given percentages are the percentages of CD3-CD4+ T-cells which express CD7

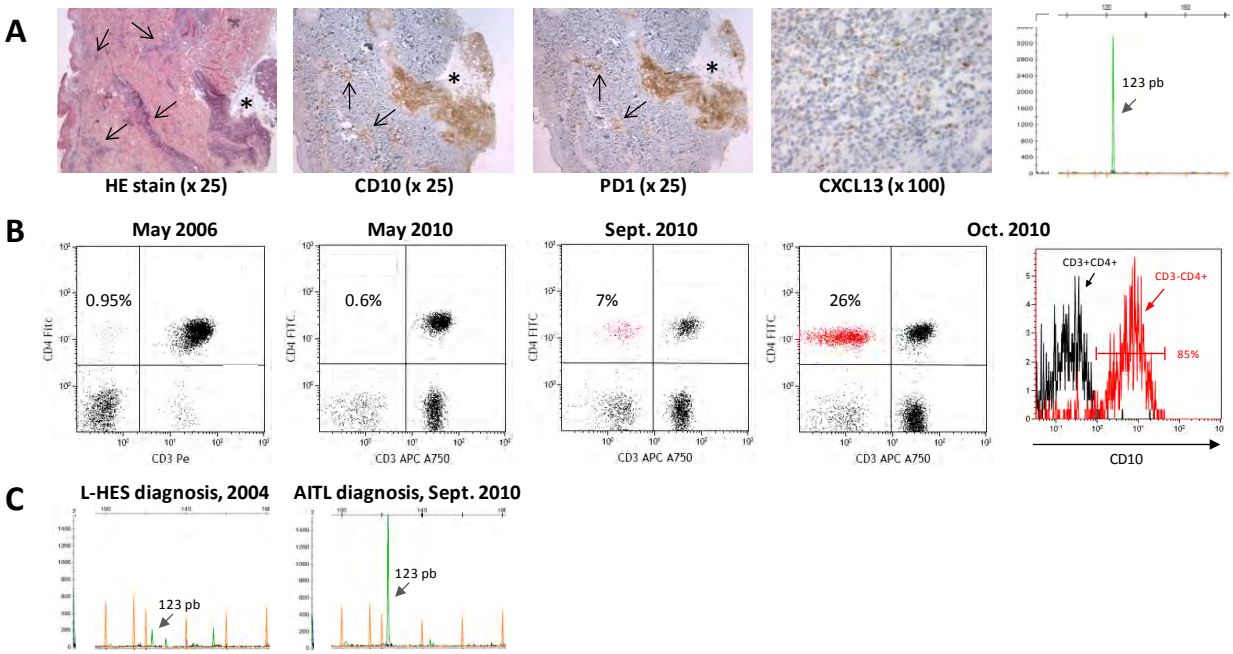


Figure S7. Immunophenotyping and histopathological features of AITL in patient P16.

Skin biopsy performed at AITL diagnosis shows large lymphoid infiltrates in all the dermis (black arrows), and in the hypodermis (*). Lymphoid cells are positive for CD10 and T-follicular helper markers PD1 and CXCL13. A clonal TCRγ rearrangement is found in skin lesions (A). Circulating CD3-CD4+ T-cell count increased at AITL diagnosis and 85% of them were CD10-positive (B) (not available in previous samples). The same circulating clonal T-cells were present in 2004 at L-HES diagnosis and in 2010 at AITL diagnosis (C).

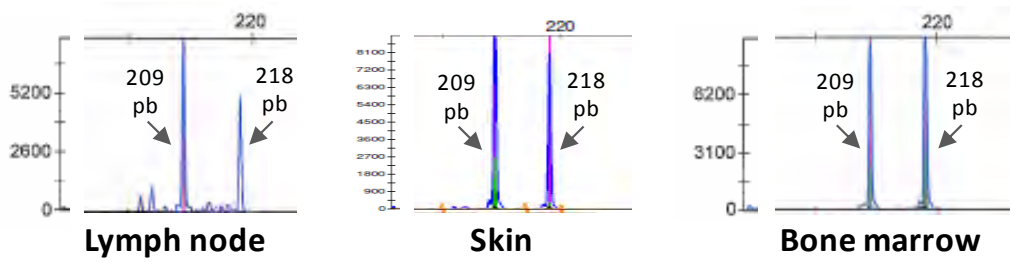


Figure S8. TCRγ rearrangement study in various biopsy at AITL diagnosis in patient P4