

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

Guillaume Lefèvre,^{1,2*} Marie-Christine Copin,^{3*} Christophe Roumier,⁴ Hélène Aubert,⁵ Martine Avenel-Audran,⁶ Nathalie Gardel,⁴ Stéphanie Poulain,⁴ Delphine Staumont-Sallé,⁷ Julien Seneschal,⁸ Gilles Salles,⁹ Kamel Ghomari,¹⁰ Louis Terriou,² Christian Leclech,⁶ Chafika Morati-Hafsouai,¹¹ Franck Morschhauser,¹² Olivier Lambotte,¹³ Félix Ackerman,¹³ Jacques Trauet,¹ Sandrine Geffroy,⁴ Florent Dumezy,⁴ Monique Capron,¹⁴ Catherine Roche-Lestienne,¹⁵ Alain Taieb,⁸ Pierre-Yves Hatron,² Sylvain Dubucquoi,¹ Eric Hachulla,^{1,2} Lionel Prin,¹ Myriam Labalette,¹ David Launay,^{1,2} Claude Preudhomme, and ^{4**} Jean-Emmanuel Kahn,^{1,16**} on behalf the French Eosinophil Network

¹Institute of Immunology, French Eosinophil Network and Research Unit EA2686, Lille University Hospital, Université Lille Nord de France, Lille; ²Department of Internal Medicine - Clinical Immunology Unit and Research Unit EA2686, Lille University Hospital, Université Lille Nord de France, Lille; ³Institute of Pathology and CNRS Unit Research UMR 8161, Lille University Hospital, Université Lille Nord de France, Lille; ⁴Institute of Hematology and Inserm Unit U837, Lille University Hospital, Université Lille Nord de France, Lille; ⁵Department of Dermatology, Nantes University Hospital, Nantes; ⁶Department of Dermatology, Angers University Hospital and UNAM University, Angers; ⁷Department of Dermatology, Lille University Hospital, Université Lille Nord de France, Lille; ⁸Department of Dermatology and Pediatric Dermatology, National Reference Center for Rare Skin Diseases and Inserm Unit Research U1035, Bordeaux University Hospital, Bordeaux; ⁹Department of Hematology, Lyon Sud University Hospital, Hospices Civils de Lyon, Lyon 1 University, Bron; ¹⁰Department of Onco-Hematology, Beauvais Hospital; ¹¹Department of Internal Medicine, Annecy Hospital, Annecy; ¹²Department of Internal Medicine, Bicêtre University Hospital - APHP, Paris Sud XI University, Le Kremlin-Bicêtre; ¹³Department of Hematology, Lille University Hospital, Université Lille Nord de France, Lille; ¹⁴Inserm Unit Research U995, Lille University Hospital, Université Lille Nord de France, Lille; ¹⁵Institut de Génétique Médicale, Inserm Unit U837, Lille University Hospital, Université Lille Nord de France, Lille; and ¹⁶Department of Internal Medicine, Foch Hospital and Versailles-Saint-Quentin-en-Yvelines University, Suresnes, France

*GL and M-CC and **CP and J-EK each contributed equally to this work.

ABSTRACT

The CD3⁺CD4⁺ lymphoid variant of hypereosinophilic syndrome is characterized by hypereosinophilia and clonal circulating CD3⁺CD4⁺ T cells. Peripheral T-cell lymphoma has been described during this disease course, and we observed in our cohort of 23 patients 2 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 10 of 12 CD3⁺CD4⁺ L-HES patients, in lymph nodes (n=4 of 4 patients), in skin (n=9 of 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 2 fresh skin samples confirmed the presence of CD3⁺CD4⁺ T cells. Clonal T cells were detected in at least one tissue in 8 patients, including lymph nodes (n=4 of 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 CD2^{hi} (n=9 of 14), CD5^{hi} (n=12 of 14), and CD7⁻ (n=4 of 14) or CD7^{low} (n=10 of 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-cell 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 tissue 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) more than 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 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 CD3^{low}CD4⁺ T cells have also been detected by flow cytometry in various periph-

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Correspondence: guillaume.lefevre@chru-lille.fr

eral T-cell lymphomas (PTCL) but mainly in angio-immunoblastic 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

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	Organ 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, lacrimal glands, parotid, subcutaneous nodules	2011, 2013	2013	Lacrimal gland (1990)	2013	CS, CSA, MTX, Phototherapy, peg-IFN α	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, IFN α , 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, IFN α , 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, joints	2010 (AITL)		BM (2004: L-HES; 2010: AITL)	2010	CS, IFN α	8	Died (AITL, 2010)

^aDisease 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; IFN α : interferon α ; MEPO: mepolizumab; MTX: methotrexate; HU: hydroxycarbamide; IM: imatinib; aSCT: allogeneic stem cell 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			Patterns of T cell infiltration		Increased and/or disorganized FDC CD23 ⁺ meshwork ²					Immunophenotypic profile of T-cells ²				Clonal TCR _γ rearr. ³
		Size	Nucleus	Cytopl. ¹	Level of effacement of architecture by infiltrates	Respect of peripheral cortical sinuses ²	FDC CD23 ⁺ meshwork ²	HEV ²	Eos. ²	Plasm. ²	EBV ⁺ B cell 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,	++	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; ²0: 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 TCR_γ rearrangement is indicated as present ("n.a."). 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; TCR 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-cell infiltration							Immunophenotypic profile of T cells ³				Clonal TCR _γ rearr. ⁴		
		Size	Nucleus	Cytopl. ²	Epid. ³	->Epid. ³	S/D	N/D	PV ³	PA ³	Hypod. ³	Eos. ³	Plasm. ³	CD3 ⁺ CD4 ⁺ CD5 ⁺	CD10		PD1	CXCL13
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	+	+	++	+	+/-	+	+	+	+	+

¹Skin lesions in L-HES patients were pruritic papulo-nodular in patients 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 ³0: 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 TCR rearrangement is indicated as present ("n.a") 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 TCR_γ 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; TCR rearr: T cell Receptor 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.

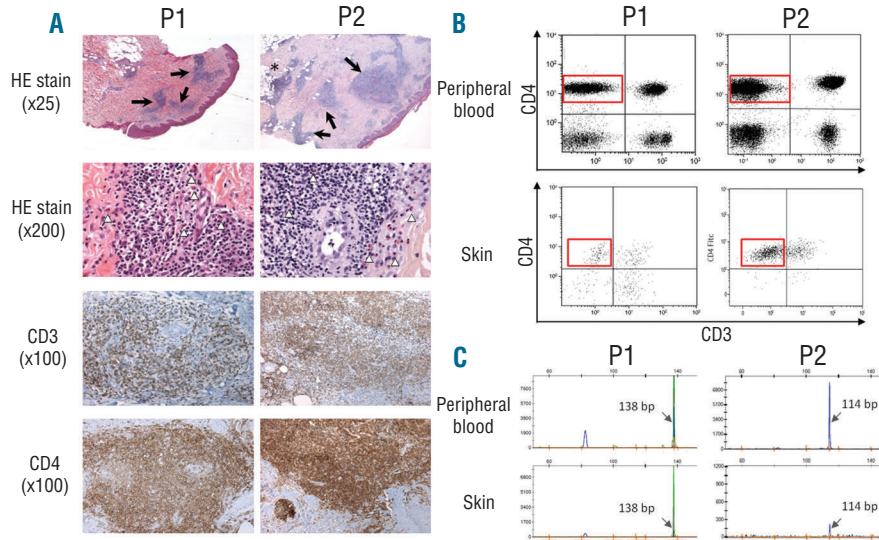


Figure 1. Skin histopathology in CD3⁺CD4⁺ L-HES. Hematoxylin and Eosin (HE) staining of these two representative skin biopsies (Patients P1 and P2) reveal dense nodular, periadnexal and perivascular infiltrates (black arrows), a hypodermis infiltrate in P2 (*). Lymphoid cells are small to medium-sized, 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 (data not shown). Flow cytometry confirms the presence of CD3⁺CD4⁺ T cells in skin lesions (B). The same clonal TCR rearrangement is detected in skin and blood samples (C).

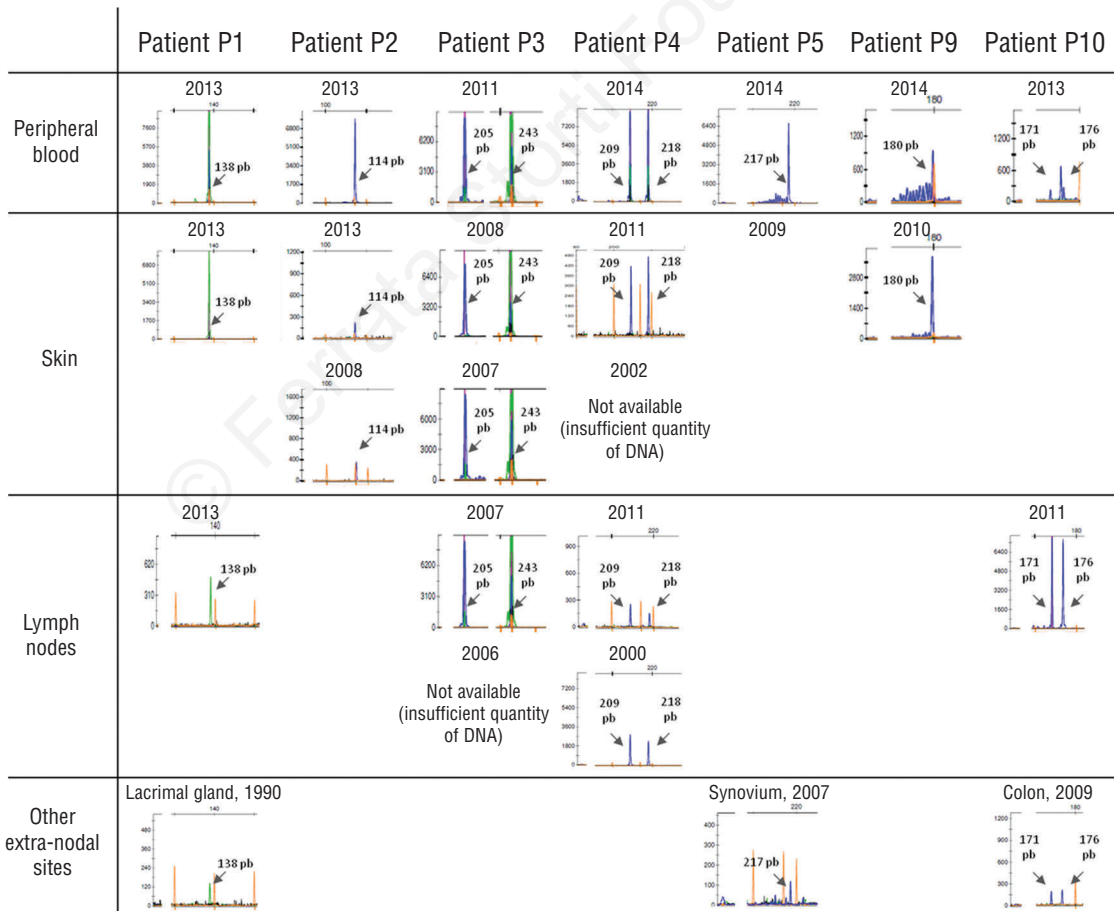


Figure 2. Persistence of clonal T cells in peripheral blood and various tissues CD3⁺CD4⁺ L-HES patients. Comparison of TCR rearrangement (γ1-10 and γ9-11 family genes) between the most recent peripheral blood sample and various tissues biopsies in 7 representative CD3⁺CD4⁺ L-HES patients. Note the spatial 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.

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 up-dated consensus definitions.¹ Main clinical characteristics are summarized in Table 1. For the 7 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 Declaration of Helsinki.

Ten of these patients had bone marrow biopsies at CD3⁺CD4⁺L-HES diagnosis in order to exclude a T-cell lymphoma (Patients

P2-4, P8, P9, P11, P12, P14-16) (*data not shown*).

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

Finally, Patients 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 a 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-cell count (28 G/L), lymph node histological examination confirmed lymphoid reactive hyperplasia. As he was in really good

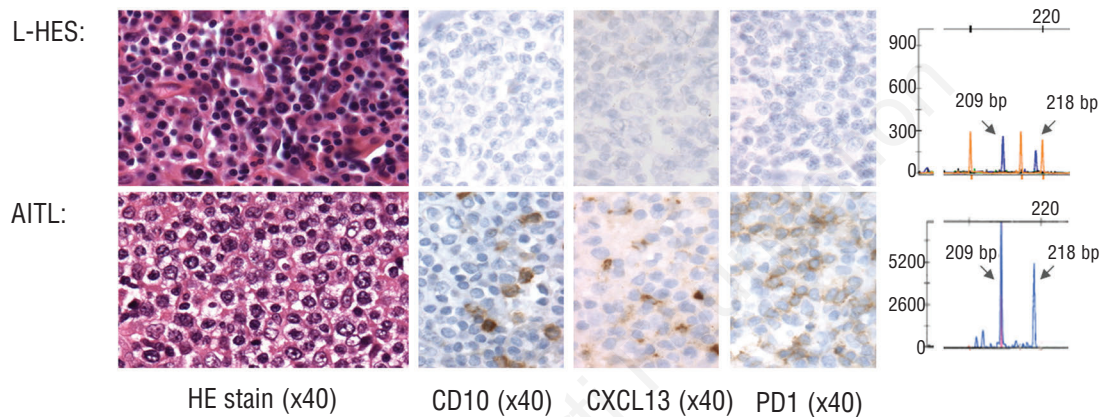


Figure 3. Comparison of cytological characteristics of lymphoid cells in CD3⁺CD4⁺L-HES and at AITL diagnosis in patient P4. In context of CD3⁺CD4⁺L-HES (lymph node biopsy performed during follow up in 2011), lymphoid cells were monomorphic, small- to medium-sized, with irregular nucleus and scarce cytoplasm (HE: Hematoxylin & 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 CD10, CXCL13 and/or PD1-positive cells are found. In both cases, the same clonal TCR γ rearrangement was detected.

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 TCR γ rearr.
				Size	Nucleus	Cytopl. ²					CD3 ⁺ CD4 ⁺ CD5 ⁺	CD10	PD1	CXCL13	
P1	L-HES 1990	Lacrimal gland	+++	s/m	irregular, dense	+	Dense lymphoid infiltrates, partial effacement of lacrimal 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												
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 2011	Digestive tract	0					0							

¹Patients 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); ²0: 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; TCR γ rear; Tcell receptor γ rearrangement; L-HES: lymphoid variant of hypereosinophilic syndrome; s/m: small-to-medium size.

health and was slightly embarrassed by his symptoms, the diagnosis of T-cell lymphoma was not retained and he did not receive any treatment, except 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 presented in 2002 and were pruritus and/or urticarial plaques, polyarthritis. L-HES diagnosis was made in 2004 on the association of HE (4.5 G/L), clonal TCR γ rearrangement and really small but persistent CD3⁺CD4⁺ circulating T-cell subset. Corticosteroids and interferon-alpha given as a corticosteroid-sparing treatment have improved symptoms and normalized eosinophil count. All treatments were stopped in October 2009. In July 2010, a diagnosis of AITL was made after an amoxicillin-induced rash, followed by systemic manifestations including fever, lips angioedema and polyarthritis. AITL was never controlled despite appropriate chemotherapy (alemtuzumab and CHOP chemotherapy, vinblastine) and the patient died four 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 investigation was carried out similar to those performed for T-cell lymphomas. Nine patients had at least one available skin biopsy during their follow up, 4 patients had at least one lymph node biopsy or a suspicion of lymphoma, 5 had digestive biopsies (available in 4 patients), one patient had a synovial biopsy, one patient had a lacrimal 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 a description of atypical lymphoid cells, pattern of infiltration and presence of other cellular types in the infiltrates (eosinophils and notably plasmocytes). Deparaffinized tissue sections were stained for CD3, CD4, CD5, CD8, CD10, CD20, CD56, PD1, CXCL13. *In situ* hybridization for Epstein-Barr virus (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 (TCR γ) 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 TCR γ rearrangement 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, 15 were newly collected (n=13), or re-analyzed (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 more than 95% or less than 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. TCR γ rearrangement analysis was also performed in peripheral blood, as previously described.²⁷ Analysis was carried out 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 4 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 maintained 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

Table 5. Bone marrow histopathology in 2 CD3⁺CD4⁺ L-HES patients who developed AITL .

Patients	Diagnosis and available samples	Lymphoid infiltrate	Morphology of atypical T cells			Pattern of T-cell infiltration	Eos. ²	Plasm. ²	EBV ⁺ B cells ²	Immunophenotypic profile of T cells ²				Clonal TCR γ 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 paratrabeular	+	+	0	+	+	+	+	+
P16	L-HES (2004)	0					+++							n.a
	AITL (2010)	+++	s/m	irregular, dense	+	N/D, perivascular and paratrabeular	++	+/-	0	+	+	+	+	+

¹+ scarce cytoplasm, ++ quiet abundant cytoplasm, +++ abundant cytoplasm; ²0: 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 γ rearr: Tcell receptor γ rearrangement; 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.

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 and *Online Supplementary Figure S1*). Lymphoid infiltrates were composed of atypical monomorphic small- to medium-sized 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 TCR γ rearrangement was detected in all patients (Table 2). In one case (P10), a 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 (*Online Supplementary Figure S2*). Since then, the patient has been transiently treated with cyclosporin A for a severe psoriasis, and kept in good health for three years under corticosteroid treatment alone (prednisone 10 mg/d).

Skin histopathology in CD3⁺CD4⁺ L-HES

A superficial and/or deep dermis inflammatory infiltrate without epidermotropism was observed in all 9 cases. No patient had epidermis involvement and 2 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 of 6) (*Online Supplementary 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, 2 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). A TCR γ 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 TCR γ rearrangement was detected in at least one skin biopsy in 6 of 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 4 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 that partially destroyed 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 (*Online Supplementary Figure S4*). A clonal TCR γ rearrangement was detected in all 3 biopsies with lymphoid infiltrates (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 (*Online Supplementary Figure S5*). In comparison to conventional CD3⁺CD4⁺ T cells, CD2 and CD5 expressions were identical or increased, CD7 expression was absent (n=4 of 14) or partial/diminished (n=10 of 14). CD10 expression was never detected (n=0 of 14) (*Online Supplementary Table S1* and *Online Supplementary Figure S6*).

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 TCR γ rearrangement was observed in the last blood sample and in at least one tissue for 8 of 10 patients and in several biopsies for 5 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 2 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 TCR γ 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 4's lymph node sample 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 TCR γ 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*), a 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 and *Online Supplementary Figure S7A*). There were rare large B cells and no EBV-positive B cells. In both AITL/L-HES patients, a clonal TCR γ rearrangement was detected in skin biopsy.

Bone marrow

Large CD10 and TFH marker-positive lymphoid infiltrates were found in the bone marrow biopsies of P4 and P16. 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 TCR γ 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-cell subset increased at AITL diagnosis and 85% of cells were CD10-positive (unfortunately this parameter was not available in previous immunophenotypic analysis) (*Online Supplementary Figure S7B*).

TCR γ rearrangement study

In Patient P4, the same TCR γ 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 *Online Supplementary Figure S8*). In Patient P16, the same clonal TCR γ 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 (*Online Supplementary 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 2 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 2 fresh skin samples by flow cytometry confirmed that CD4⁺ T cells predominantly lacked CD3 membranous expression. We can suppose that T cells are responsible for secondary tissular eosinophilia, as suggested by a previous report that showed IL-5 production by skin aberrant T cells in 2 cases.²¹ On the other hand, the rare cases of isolated tissular eosinophilia (without any detected abnormal lymphoid population) may be explained by blood eosinophil migration into tissues, like digestive tract for Patients P11 and P14, or less likely, by very small amounts of clonal T cells in tissue. Furthermore, the same clonal TCR γ 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 that 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 2 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. In agreement with this, CD10 and TFH markers were detected in biopsies at AITL diagnosis in both AITL/L-HES Patients P4 and P16. Re-analysis 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 morphological features were in favor of 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 either CD3⁺CD4⁺ L-HES patient, or 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.^{5,6,19-21} 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-cell 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-cell infiltrates. We also reported the 2 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.

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