

Clinical Communications

Assessment of T-cell polarization on the basis of surface marker expression: Diagnosis and potential therapeutic implications in lymphocytic variant hypereosinophilic syndrome

Emmanuel Ledoult, MD^{a,b,c}, Matthieu Groh, MD^{d,e}, Jean-Emmanuel Kahn, MD, PhD^{d,e}, Jacques Trauet, Eng^{a,b}, Jean-David Bouaziz, MD, PhD^f, Aurélie Caristan, MD^g, Vincent Cottin, MD, PhD^h, Sylvain Dubucquoi, MD, PhD^{a,b}, Nicolas Etienne, MD^{c,d}, Cécile Golden, MDⁱ, Perrine Guillaume-Jugnot, MD^j, Éric Hachulla, MD, PhD^{b,c,d}, David Launay, MD, PhD^{b,c,d}, Irène Machelart, MD^{d,k}, Adèle De Masson, MD, PhD^f, Thomas Molinet, MD^l, Chafika Morati-Hafsaoui, MD^{d,m}, Marie Puget, MDⁿ, Mathilde Roumier, MD^e, Louis Terriou, MD^{b,c,d}, Bertrand Meresse, PhD^b, Arnaud Dendooven, Eng^{b,d}, Marie-Christine Copin, MD, PhD^o, Romain Dubois, MD^o, Myriam Labalette, MD^{a,b}, and Guillaume Lefèvre, MD, PhD^{a,b,c,d}, for the CEREO—French National Reference Center for Hypereosinophilic Syndromes

Clinical Implications

- Lymphocytic variant hypereosinophilic syndrome (LHES)-associated CD3⁺CD4⁺ T cells in blood homogeneously express chemokine receptors associated with T_H2 polarization (CCR₆⁺CCR₄⁺)—even if the patients are treated with steroids. New treatment strategies such as CRTh₂ antagonists and anti-CCR₄ antibody could be considered in severe LHES.

TO THE EDITOR:

After having ruled out the most frequent causes of both reactive eosinophilia (eg, helminthic, drug-induced, and paraneoplastic conditions) and clonal eosinophilia, the etiological workup for hypereosinophilic syndrome (HES) usually comprises a screen for the lymphocytic variant (LHES).^{1,2} In LHES, the overproduction of IL-5 by T_H2-polarized T cells leads to an indolent, lymphoproliferative disorder with persistent eosinophilia and subsequent organ damage.³⁻⁵ A clonal T-cell receptor (TCR) rearrangement is frequently observed but may remain undetected when the aberrant T-cell populations are small.⁵ Of the abnormal T-cell phenotypes reported in LHES, CD3⁺CD4⁺ T cells are most frequently described; their ability to overproduce IL-5 *in vitro* has been extensively documented.^{3,4} However, the detection of IL-5 overproduction is not performed on a routine basis in clinical laboratories and is unreliable in patients treated with glucocorticoids. Hence, there is an unmet need for the implementation of new diagnostic biomarkers of LHES.

During T-cell polarization, distinct lineage-specifying transcription factors induce various functional properties, as characterized by specific cytokine production and chemokine receptor expression profiles.⁶ Hence, human T_H2 cells (producing IL-4, IL-13, and IL-5) typically express CCR₄ in the absence of CCR₆, whereas a small subset of terminally differentiated T_H2 cells also express a chemoattractant receptor homologous molecule expressed on T_H2 cells (CRTh₂) and high levels of GATA3.⁶ IFN γ -producing T_H1 cells are associated with a CCR₆⁺CCR₄⁺CxCR₃⁺ phenotype, whereas T cells producing both IFN γ and T_H2 cytokines (T_H2/T_H1 cells) are reportedly involved in immunity against viral infections.⁶

Here, we showed that LHES-associated CD3⁺CD4⁺ T cells express T_H2-associated chemokine receptors (CCR₆⁺CCR₄⁺). We also evidenced an association between CRTh₂ expression, angioedema, and skin manifestations.

All blood samples from patients with CD3⁺CD4⁺ LHES referred to the French National Reference Center for HES (CEREO) between May 2018 and May 2019 were screened for eligibility: all of them met the diagnostic criteria for HES¹ and had significant numbers of aberrant CD3⁺CD4⁺ T cells ($\geq 0.5\%$ of persistent peripheral T cells).^{3,5}

Flow cytometry phenotyping was performed on whole blood. Once aberrant CD3⁺CD4⁺ T-cell populations had been evidenced (using the method described previously),^{4,5} additional cell surface markers were investigated to better characterize the cells' polarization.⁶ T_H17, T_H1/T_H17, T_H2, T_H2/T_H1, and T_H1 lineages were defined by CCR₆⁺CCR₄⁺/C_xCR₃⁺, CCR₆⁺CCR₄⁺/C_xCR₃⁺, CCR₆⁺CCR₄⁺CXCR₃⁺, and CCR₆⁺CCR₄⁺CxCR₃⁺ expression patterns, respectively.⁶

Comparisons were made using a *t*-test for normally distributed quantitative variables, the Mann-Whitney rank test for non-normally distributed quantitative variables, and Fisher's exact test for categorical variables. All statistical tests were 2-tailed, and the threshold for statistical significance was set to $P < .05$.

We measured the polarization of LHES-associated CD3⁺CD4⁺ T cells from 20 patients (11 women). At the time of the evaluation, the donors' mean $\pm$ standard deviation age was 48.4 ± 15.7 , and the median [interquartile range] disease duration was 2.9 [0.6; 8.0] years. Serum IgE and IgM levels were increased in 12 of 17 (71%) and 5 of 15 (33%), respectively. Four of the 5 patients with increased serum IgM levels also suffered from episodic angioedema, and by so, fulfilled criteria for Gleich's syndrome. Eight patients were being treated with topical or oral steroids, 3 were receiving mepolizumab, and 6 were not receiving any treatment for HES. The median absolute eosinophil count (G/L) was 1.0 [0.4; 2.4], and the median proportion of the CD3⁺CD4⁺ T-cell subset (as a percentage of total lymphocytes) was 3.7% [2.5; 14.4] (Table 1). All LHES-associated CD3⁺CD4⁺ T-cell subsets expressed T_H2-polarization markers (CCR₆⁺CCR₄⁺), contrary to the corresponding subsets from 3 controls who had peripheral CD3⁺CD4⁺ T cells without eosinophilia (Figure 1; Figure E1, available in this article's Online Repository at www.jaci-inpractice.org). In the same patients with LHES, the

TABLE I. Clinical and laboratory features of patients with λ HES with CD3-CD4⁺ T cells

Pat	Sex	Age, y	Diagnosis			Follow-up		Present evaluation				
			AEC _{max}	%LC	TCR rearr	Organ involvement	Previous drug treatments	Disease duration, y	AEC	%LC	Polarization markers	Current drug treatments
P1	F	56	3.4	9.0	+	Pruritus; coronary and carotid aneurysms; LN	CS MEPO	9.2	0.2	3.3	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ^{80%+}	MEPO
P2	M	37	7.0	2.8	–	Diarrhea; abdominal pain*	None	0.3	6.2	2.3	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ⁺	None
P3	F	71	2.0	56.0	+	Eczema-like; eosinophilic gastroenteritis	CS, IFN MEPO	4.9	0.4	29.3	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ^{87%+}	MEPO
P4	F	33	2.5	0.6	–	Eczema-like	CS	2.2	1.9	0.4	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ^{50%+}	None
P5	F	60	12.0	25.0	+	Pruritus; eosinophilic gastroenteritis	CS MEPO	10.8	0.4	15.8	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ^{55%+}	MEPO
P6	M	56	17.5	58.0	+	EAE; Eczema-like	CS	3.3	0.3	10.0	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ⁺	CS
P7	M	74	1.3	2.0	–	Eczema-like	tCS	0	1.0	2.0	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ⁺	tCS
P8	F	40	2.7	24.3	+	Eczema-like; tenosynovitis	CS	8.7	0.1	4.0	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ⁺	None
P9	M	68	2.2	38.8	+	Urticaria, prurit	CS, IFN	11.0	2.9	58.1	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ⁺	CS
P10	M	57	5.3	8.5	+	Eczema-like; LN; tenosynovitis	tCS	1.0	2.6	9.2	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ^{86%}	tCS
P11	M	55	3.7	40.0	+	Asthenia; sweats; hot flushes	CS	2.1	0.2	5.0	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ⁻	CS
P12	M	55	8.0	15.0	+	EAE [†]	CS	8.9	1.5	1.4	CCR ₆ ⁻ CCR ₄ ^{85%+} CxCR ₃ ⁻ CRTh ₂ ⁻	CS
P13	F	40	16.8	4.3	+	EAE [†] ; pruritus; LN; SMG	CS	2.4	2.5	25	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁻ CRTh ₂ ⁻	CS
P14	F	59	2.0	2.0	–	EAE	CS	3.4	0.7	3.0	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁺ CRTh ₂ ⁻	NA
P15	M	45	7.2	10.0	NA	Nonepisodic angioedema; eosinophilic myositis with fasciitis	CS, AZA, MTX, CSA	5.9	1.2	3.3	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁺ CRTh ₂ ⁻	None
P16	F	17	15.0	20.0	–	Urticaria; EAE [†]	CS	1.8	6.0	3.4	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ⁺ CRTh ₂ ⁻	None
P17	F	38	0.9	21.7	+	Eczema-like	tCS	0	0.9	21.7	CCR ₆ ⁻ CCR ₄ ⁺ CxCR ₃ ^{80%+} CRTh ₂ ^{na}	tCS

P18	F	49	20.0	2.6	—	EAE [†]	CS	0.5	0.9	2.9	CCR ₆ ⁺ CCR ₄ ⁺ C _α CR ₃ ⁺ CRTh ₂ ^{na}	NA
P19	F	18	3.8	1.2	—	Asthenia, paresthesia	CS	4.0	1.0	1.3	CCR ₆ ⁺ CCR ₄ ⁺ C _α CR ₃ ^{50%} CRTh ₂	CS
P20	M	40	4	4.3	—	EAE	None	0	0.7	4.3	CCR ₆ ⁺ CCR ₄ ⁺ C _α CR ₃ ^{21%} CRTh ₂	None

%L/C, CD3⁺CD4⁺ T cells as a percentage of total lymphocytes; AEC, absolute eosinophil count (G/L); AZA, azathioprine; CS, oral corticosteroids; CSA, cyclosporine; EAE, episodic angioedema with eosinophilia; *l*HES, lymphocytic variant hyper-eosinophilic syndrome; LV, lymph node enlargement; MEPO, mepolizumab; MRI, magnetic resonance imaging; MTX, methotrexate; NA, not available; Pat, patient; SMG, splenomegaly; TCR *rear*, clonal TCR rearrangement in whole blood ("+" or "—" respectively indicate that a clonal rearrangement was detected or not); rCS, topical corticosteroids only.

Disease duration (in years) was defined as the time between the diagnosis of *l*HES and the current evaluation.

*No eosinophilic infiltrate was found on digestive tract biopsies. Cardiac MRI showed nonspecific pericardial and myocardial abnormalities. There was no chest pain but there were no abnormalities (except a mild mitral valvulopathy) on the ECG or on ultrasound. The serum troponin level was normal.

[†]In these patients, the association of EAE and increased serum IgM level defined Gleich's syndrome.

CCR₄⁺CCR₆⁺ subset ranged only from 6.5% to 35% in peripheral CD3⁺CD4⁺ T cells.

Next, our assessment of C_αCR₃ expression on CD3⁺CD4⁺ T cells further differentiated between patients with a (C_αCR₃⁺) T_H2/T_H1 subset (n = 6) and those without (n = 14). The 2 groups of patients differed solely with regard to the serum total IgE level, which was more often elevated in patients with the (C_αCR₃⁺) T_H2 phenotype (*P* = .002). We then measured CRTh₂ expression on CD3⁺CD4⁺ T cells from 18 of the 20 patients. Interestingly, episodic angioedema (*P* = .013) were more frequent in the CRTh₂⁺ group (defined by both CRTh₂⁺ T_H2 [CCR₆⁺CCR₄⁺C_αCR₃⁺CRTh₂⁺; n = 4] and CRTh₂⁺ T_H2/T_H1 [CCR₆⁺CCR₄⁺C_αCR₃⁺CRTh₂⁺; n = 4] subsets) than in the CRTh₂⁺ group (defined by CCR₆⁺CCR₄⁺C_αCR₃⁺CRTh₂⁺; n = 10) (Figure 1; Table E1, available in this article's Online Repository at www.jaci-inpractice.org). Conversely, other skin manifestations (mostly eczema-like lesions, pruritus, etc.) were more frequent in the CRTh₂⁺ group (*P* = .013), suggesting 2 potential different patterns of the disease.

The correlation between cytokine secretion and chemokine receptor expression is well known.^{6,7} Here, we demonstrated for the first time that *l*HES-associated CD3⁺CD4⁺ T cells homogeneously express chemokine receptors associated with T_H2 polarization (CCR₆⁺CCR₄⁺)—regardless of whether the patients were treated with steroids or not. We also observed a higher frequency of angioedema in the CRTh₂⁺ group and a higher frequency of other skin manifestations in the CRTh₂⁺ group.

Interestingly, CCR₄⁺ is a receptor for the C–C chemokine ligand 17 (CCL17, also known as thymus- and activation-regulated chemokine), levels of which are elevated in *l*HES.³ Furthermore, the CD3⁺CD4⁺ T cells had a mixed T_H2/T_H1 phenotype in some patients, in line with the previous reports on some patients with *l*HES-associated CD3⁺CD4⁺ T cells that secreted IL-5 and IFN_γ *in vitro*.³

Our study had a number of limitations. We have not yet investigated patients with *l*HES with CD3⁺CD4⁺CD8⁺TCR^{αβ}⁺ or CD3⁺CD4⁺CD7⁺ T cells: further evaluations of these markers in these rare suspected cases of *l*HES will indicate the potential involvement of other T-cell subsets in the onset of eosinophilia. We are also currently performing a prospective study of whether C_αCR₃ and CRTh₂ expression in a given patient persists over time, and if steroids could have an influence on them.

Better knowledge of cell surface marker expression on CD3⁺CD4⁺ T cells may also have therapeutic consequences. Indeed, one could consider using CRTh₂ antagonists to target both CRTh₂⁺ CD3⁺CD4⁺ T cells and eosinophils, which also express CRTh₂.⁷ Moreover, a monoclonal anti-CCR₄ antibody has demonstrated its efficiency in the treatment of malignant CD4⁺ T-cell lymphoproliferative disorders (including adult T-cell lymphoma/leukemia and cutaneous T-cell lymphoma),⁸ and in a recent case of CCR₄⁺ angioimmunoblastic T-cell lymphoma (AITL). Because the latter is also a complication of CD3⁺CD4⁺ *l*HES, mogamulizumab could be considered for the treatment of both severe refractory *l*HES and *l*HES-related AITL.⁹ Overall, our findings suggest that (1) a novel diagnostic approach for suspected *l*HES (confirmation of the T_H2 polarization) could be easily evaluated in other aberrant T-cell subsets (CD3⁺CD4⁺CD8⁺TCR^{αβ}⁺ or CD3⁺CD4⁺CD7⁺ T cells), and (2) new treatment strategies could be considered (such as

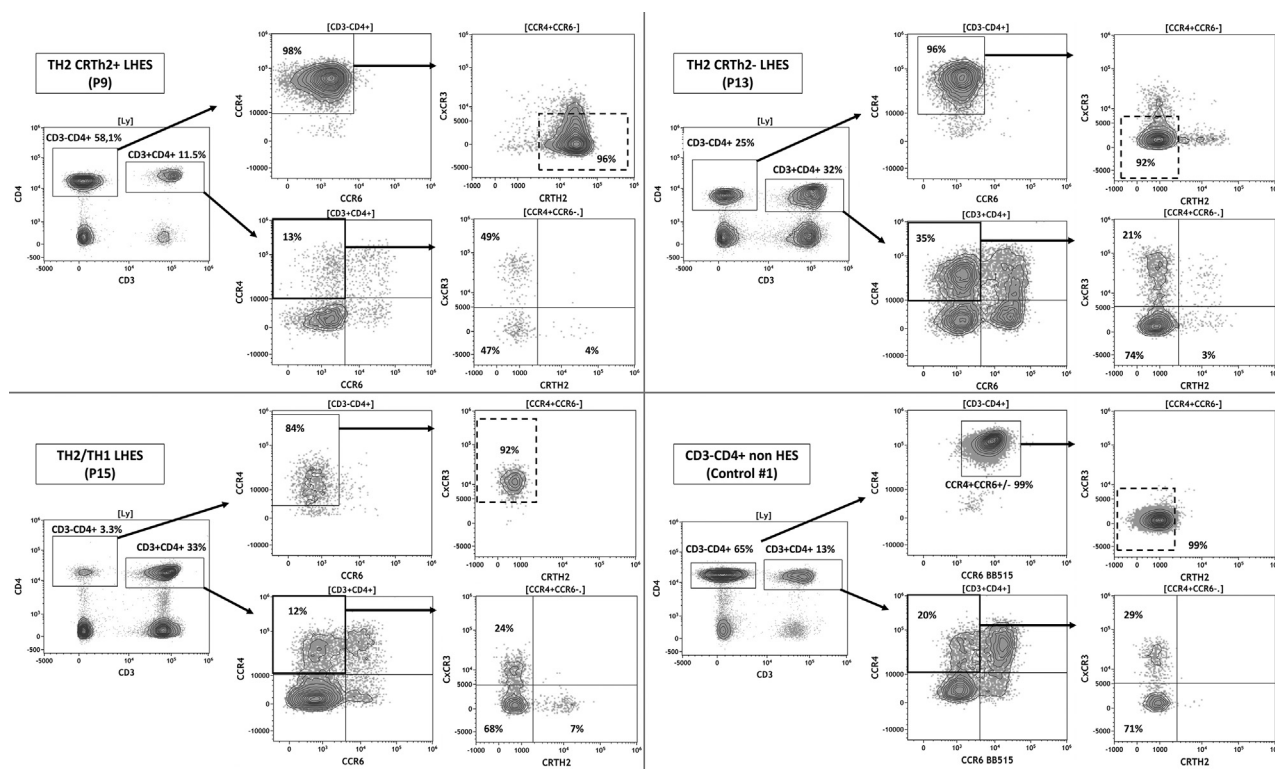


FIGURE 1. T_H2 polarization markers on L HES-associated $CD3^+CD4^+$ T cells. Multiparameter flow cytometry showing a common T_H2 -polarization phenotype, based on $CCR6^+CCR4^+$ expression in 3 representative patients with L HES with $CD3^+CD4^+$ T cells. One patient with aberrant $CD3^+CD4^+$ T cells but no eosinophilia is presented as control. Right panels: gating of $CD3^+CD4^+$ cells and $CD3^+CD4^+$ on lymphocyte cells; middle: expression of T_H2 -associated markers $CCR6^+$ and $CCR4^+$; right: expression of $CXCR3$ and $CRTH2$ on $CCR4^+CCR6^+$ cells. L HES, Lymphocytic variant hypereosinophilic syndrome.

$CRTH2$ antagonists in severe or steroid-dependent L HES, and mogamulizumab in L HES-related AITL).

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^aInstitut d'Immunologie, CHU de Lille, Lille, France

^bUniv. Lille, U995—LIRIC—Lille Inflammation Research International Center, Lille, France

^cDépartement de Médecine Interne, CHU de Lille, Lille, France

^dCentre de Référence National des Syndromes Hyperéosinophiliques (CEREO), Lille, France

^eDépartement de Médecine Interne, Hôpital Foch, Suresnes, France

^fDépartement de Dermatologie, Hôpital Saint-Louis, Paris, France

^gDépartement de Médecine Interne, CHD Vendée, La Roche sur Yon, France

^hDépartement de Pneumologie, CHU de Lyon HCL, Lyon, France

ⁱDépartement de Médecine Interne, CH de Vesoul, Vesoul, France

^jDépartement de Médecine Interne, Hôpital la Pitié-Salpêtrière, Paris, France

^kDépartement de Médecine Interne, CHU de Bordeaux, Bordeaux, France

^lDépartement de Médecine Interne, CHU de Nancy, Nancy, France

^mDépartement de Médecine Interne, CH d'Annecy-Genevois, Epagny Metz-Tessy, France

ⁿDépartement de Médecine Interne, CH de Valence, Valence, France

^oInstitut de Pathologie, CHU de Lille, Lille, France

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Corresponding author: Guillaume Lefèvre, MD, PhD, Institut d'Immunologie, Centre de Biologie-Pathologie-Génétique, CEREO, Centre de Référence Maladies Rares des Syndromes Hyperéosinophiliques, CHRU Lille, F-59037 Lille Cedex, France. E-mail: guillaume.lefevre@chru-lille.fr.

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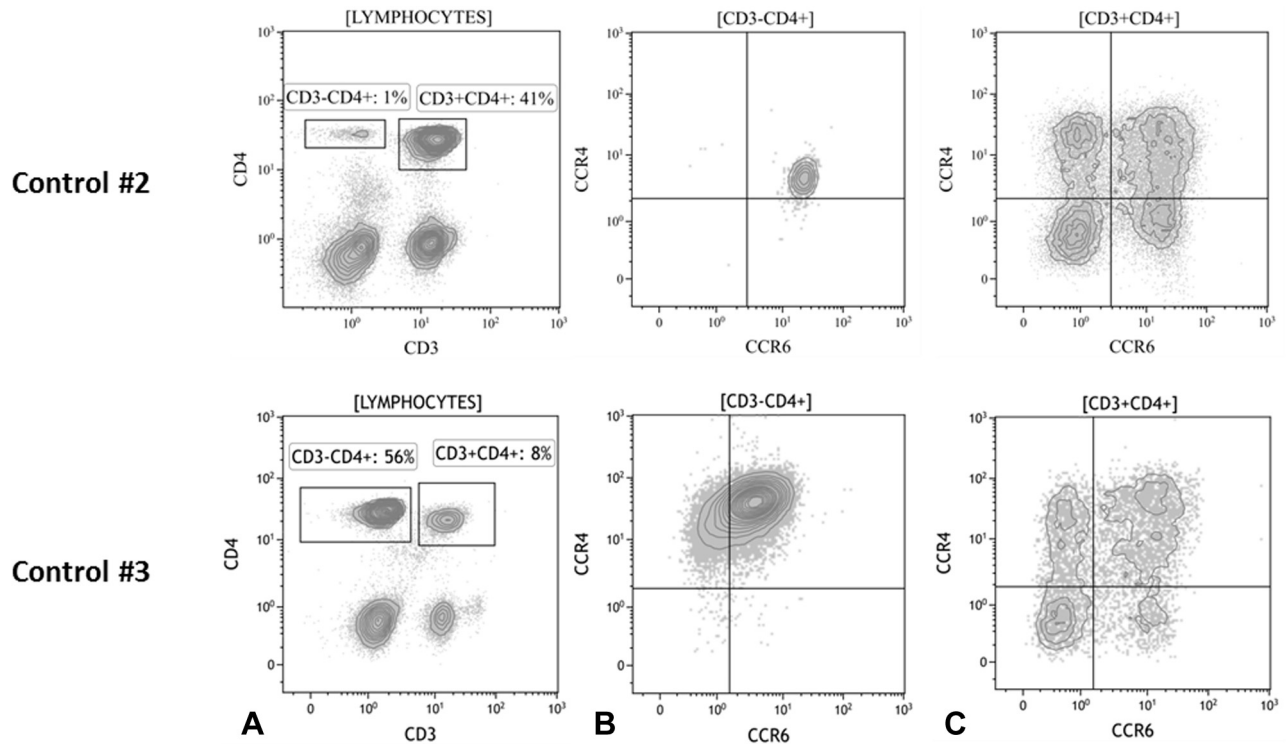


FIGURE E1. T_H2 polarization markers on $CD3^+CD4^+$ T cells in 2 control patients without eosinophilia. Control #2 is followed for myasthenia; a $CD4^+$ T-cell lymphoma not otherwise specified was diagnosed in control #3 four months after this sample. Control #1 is presented in [Figure 1](#).

TABLE E1. Characteristics of the patients with LHES as a function of their CRTh_2 status

	$\text{CRTh}_2^- \text{CD3}^- \text{CD4}^+$	$\text{CRTh}_2^+ \text{CD3}^- \text{CD4}^+$	<i>P</i>
	<i>n</i> = 8	<i>n</i> = 10	
Mean $\pm$ SD age	45.2 $\pm$ 15.4	53.4 $\pm$ 15.6	ns
Females	4/8	5/10	ns
Laboratory characteristics			
Median [IQR] current AEC	1.4 [0.5; 3.4]	0.4 [0.2; 1.7]	ns
Median [IQR] max AEC	11.5 [6.3; 17.6]	3.1 [2.1; 10.8]	ns
Median [IQR] current $\text{CD3}^- \text{CD4}^+$ (%LC)	3.4 [2.6; 10.0]	3.7 [2.1; 14.4]	ns
CxCr_3^+ subset ($\text{T}_{\text{H}}1/\text{T}_{\text{H}}2$)	4/8	0/10	0.023
Elevated serum IgE	4/7	8/10	ns
Elevated serum IgM	4/7	0/7	.07
Organ involvement			
EAE/non-EAE	6/8	1/10	.013
Gastroenteritis	0/8	2/10	ns
Coronary aneurysms	0/8	1/10	ns
Lymph nodes	1/8	2/10	ns
Skin	2/8	9/10	.013
Tenosynovitis	0/8	2/10	ns
Myositis with fasciitis	1/8	0/10	ns
Current treatment			
None	3/7	3/10	ns
Topical steroids only	0/7	2/10	ns
Oral steroids	4/7	2/10	ns
Mepolizumab	0/7	3/10	ns

AEC, Absolute eosinophil blood count (G/L); $\text{CD3}^- \text{CD4}^+$ (%LC), $\text{CD3}^- \text{CD4}^+$ aberrant T cells as a percentage of total lymphocytes; EAE, episodic angioedema with eosinophilia; IQR, interquartile range; LHES , lymphocytic variant hypereosinophilic syndrome; ns, not significant; SD, standard deviation.

Bold indicates statistical significance ($P < .05$).