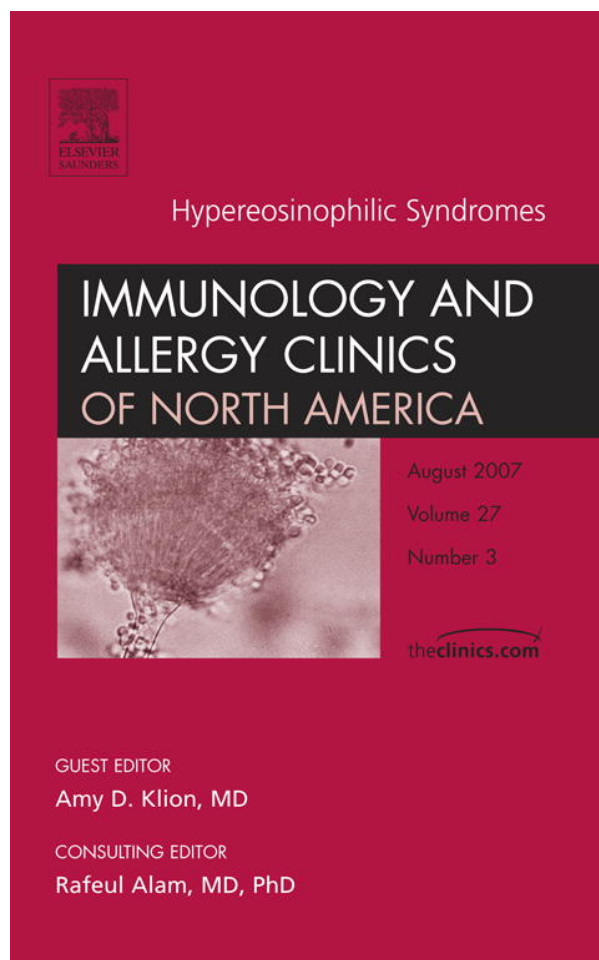




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Lymphocytic Variant Hypereosinophilic Syndromes

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The hypereosinophilic syndrome (HES) is an extremely heterogeneous disorder, in terms of clinical and biologic features, disease course and prognosis, and response to therapy. Recent work dedicated to HES pathogenesis has shown that molecular mechanisms ultimately leading to hypereosinophilia may differ significantly from one patient subgroup to another, providing a basis for clinical heterogeneity. Schematically, two types of pathogenic mechanisms have been described: either eosinophils expand clonally in the setting of a myeloproliferative disorder involving hematopoietic stem cells, with preferential eosinophilic differentiation, or eosinophils proliferate polyclonally in the setting of a cytokine-driven reactive process, with overproduction of eosinophil growth factors by T cells (lymphocytic, or L-HES) [1].

Two major discoveries in the field of fundamental immunology set the stage for suspecting that T cells could play a role in HES pathogenesis: the description of distinct helper (CD4) T cell subsets (ie, Th1 and Th2) according to their cytokine profile [2], and the identification of interleukin (IL)-5 as an essential growth and activation factor for eosinophils [3]. It rapidly emerged that Th2 cells were responsible for hypereosinophilia in allergic and parasitic disorders, through production of IL-5 [4]. Concomitant

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production of IL-4 and IL-13 by these cells explained the frequent association of hypereosinophilia with increased serum IgE levels in these disorders.

Pioneers in the field of HES reported increased IgE levels in a significant proportion of patients, and underlined the associated immunoallergic clinical profile observed in most cases [5]. Compared with HES patients with features typically encountered in myeloproliferative disease, those with increased IgE levels were less likely to have life-threatening complications of hypereosinophilia, and were more likely to respond to corticosteroids, explaining that overall disease severity was qualified as benign. Experimental evidence that T cells could be involved in HES pathogenesis was first provided by a study showing that in vitro generated T-cell clones derived from peripheral blood of HES patients were able to stimulate eosinophil colony formation when cultured in presence of bone marrow stem cells [6]. The same group later identified IL-5 as the major eosinophil colony-stimulating factor produced by their T-cell clones [7]. Shortly thereafter, in 1994, a large T-cell population bearing phenotypic abnormalities was identified by flow cytometry, in a routine blood sample from an HES patient with increased serum IgE [8]. This CD3⁺CD4⁺ T-cell subset was shown to be monoclonal, and produced high levels of the Th2 cytokines IL-4 and IL-5 in vitro, suggesting a direct role in disease pathogenesis. This first formal description of a primary T-cell disorder in a patient satisfying Chusid's [9] HES diagnostic criteria was followed by similar observations (Tables 1 and 2), including a large series of 60 patients with persistent unexplained hypereosinophilia, among whom 16 had abnormal T-cell populations [10]. In most reports, T-cell populations associated with HES bear the intriguing CD3⁺CD4⁺ surface phenotype (see Table 1); however, several other phenotypically abnormal T-cell subsets have been observed in small patient subgroups or individual cases (see Table 2). The qualifier "lymphocytic variant" HES (L-HES) was introduced recently to distinguish this proportionally significant pathogenic subgroup from idiopathic HES, and L-HES has been defined as a primitive lymphocytic disorder characterized by nonmalignant expansion of a T-cell population producing IL-5 in patients fulfilling HES diagnostic criteria.

Pathogenic mechanisms of lymphocytic hypereosinophilic syndrome

CD3⁺CD4⁺ T-cell-associated lymphocytic hypereosinophilic syndrome

Phenotypic and functional characterization of CD3⁺CD4⁺ T cells

The T-cell nature of the CD3⁺CD4⁺ subset identified by flow cytometry has been certified by several means, besides morphologic studies. First, monoclonal T-cell receptor (TCR) rearrangement patterns have been observed by Southern blot and polymerase chain reaction analysis for TCR β and TCR γ chain genes (see Table 1) [8,10–12]. Second, multicolor flow cytometry shows that they stain positively for T-cell surface antigens including CD2, CD5 (generally with a higher fluorescence intensity than normal CD3⁺CD4⁺ cells), and CD28; after permeabilization, they also stain

for intracellular CD3 ϵ and the TCR α/β framework [11,12]. Importantly, this phenotype is stable during prolonged in vitro culture, showing that loss of CD3 expression is not a transient response to chronic antigen-dependent stimulation in vivo [13]. Other phenotypic features include CD45RO positivity indicative of memory cells, loss of CD7 or CD27 surface expression, CD95 (Fas-R) positivity, and variable staining intensities for the high affinity IL-2R alpha (CD25) and HLA-DR, reflecting some degree of in vivo activation (see Table 1) [10–12]. In a few reported cases, clonal CD4 T cells have reduced CD3 expression (CD3^{dim}) rather than complete absence of this marker [10]. Decreased or absent CD3 expression on CD4 T cells has been observed in peripheral blood from patients with Sézary syndrome [14], adult T-cell leukemia-lymphoma [15], and angioimmunoblastic T-cell lymphoma [16], all malignant T-cell disorders that must be excluded before considering the diagnosis of HES.

CD3⁺CD4⁺ cells are quiescent when cultured in vitro in enriched culture medium; they show no signs of proliferation, and display a high level of spontaneous apoptosis [17]. Although these cells are generally unable to produce measurable amounts of cytokines spontaneously, low levels of IL-5 have been detected in culture supernatants in some cases [10]. Cytokine production is greatly enhanced in presence of classical mitogenic agents, such as phorbol 12-myristate 13-acetate combined with ionophore or with anti-CD28 antibodies [11]. These cells do not respond to stimulation with anti-CD3 + anti-CD28 antibodies [18], and similar observations have been reported with the lectin phytohemagglutinin [19,20], which must bind membrane CD3 to induce T-cell activation. Besides IL-5, which is consistently produced by CD3⁺CD4⁺ T cells, reported cytokine profiles show some heterogeneity (see Table 1), but overall they generally also produce the Th2 cytokines IL-4 and IL-13 [8,11,21], favoring polyclonal B-cell activation and IgE synthesis in vivo. Experimental evidence for a direct role of CD3⁺CD4⁺ T cells in associated hyper IgE in these patients has been provided in vitro. Indeed, following stimulation with pokeweed mitogen, cultured peripheral blood mononuclear cells from a patient with CD3⁺CD4⁺ cells secreted high levels of IgE into supernatants, which decreased if CD4⁺ T cells (but not if CD3⁺ T cells) were depleted [22]. Occasionally, patients have normal IgE levels, and this may be caused by the inability of their CD3⁺CD4⁺ cells to produce IL-4 and IL-13 [20]. Production of granulocyte-macrophage colony-stimulating factor may synergize with IL-5 for induction of hypereosinophilia [12]. Interestingly, CD3⁺CD4⁺ cells may also produce IL-2 and variable amounts of the Th1 cytokine interferon (IFN)- γ [11,21,23]. For investigation of IL-2 production, flow cytometry (combining surface staining for T-cell markers, with intracytoplasmic staining for cytokines) is a more reliable method than measuring concentrations in culture supernatants, caused by possible reuptake by activated CD3⁺CD4⁺ cells, which express the CD25 receptor [18].

Table 1
Hypereosinophilic syndrome patients with a CD3⁺CD4⁺ T-cell subset

Ref: Author	Gender	Age ^a FU		Clinical features		Biologic features				CD3 ⁺ CD4 ⁺ subset					Karyotype
		(yr)	(y)	Skin ^b	Other ^c	Eosino ^d /μL	Lympho ^e /μL	IgE ^f U/mL	Hyperglob ^g (mg/dL)	% gated LC ^h	Surface markers ⁱ	Clonality ^j	IL-5 ^k	Other cytokines ^l	
O'Shea et al [19]	M	27	7	"Erythem. rash"	Digit necr	5130	7400 (T cells)	518	IgM 3450	~94% (T cells)	25 ⁺ 7 ⁻	ND	ND	ND	ND
Bagot et al [41]	M	13	5	PAP, NOD	LN	19,900	5360	12,000	IgG, IgM		2 ⁺ 5 ⁺ 7 ⁻ 25 ⁻ DR ⁻	Y	ND	ND	ND
Cogan et al [8] ^m	M	30	6 ⁿ	PAP	Digit necr, LN, pulm infiltr, fever	6117	1680	2000	IgM 7200	66% (CD4)	2 ⁺ TCRα/β ⁻	Y	Y	IL4 (no IL-2, IFN-γ)	Chrom 1 break- points ⁿ
Brugnoni et al [20]	M	65	5	PAP	LN	16,600	NL	NL	IgM, IgG	38%	2 ⁺ 5 ⁺ RO ⁺ TCRα/β ⁻	N	Y	No IL-4, IL-2, IFN-γ	NL
Simon et al [10]	F	43	7	URT	—	3195	2656	738	ND	34%	5 ^{hi} 25 ⁺	Y	spon	No spon IL-4, IFN-γ	ND
	M	70	5	PAP	—	3306	2392	56	ND	17.5%	3 ⁻ 4 ^{lo} 5 ^{hi} 25 ⁺	Y	spon	ND	ND
	F	76	7	ERY	—	4532	1854	232	ND	56%	3 ^{lo} 4 ^{lo} 6 ^{lo} 7 ⁻ 25 ⁺	Y	no spon	Spon IL-4	ND
	M	55	3	ERY	—	5047	824	1147	ND	33%	3 ^{lo} 4 ⁺ 5 ^{lo} 6 ^{lo} 7 ⁻ 25 ⁺ Vβ6.7 ⁺	Y	Y	Spon IL-4	ND
Zenone et al [38]	F	32	6	EA	Smeg	2200	4810	NL	—	49%	2 ⁺ 5 ⁺ TCRα/β ⁻	Y	ND	ND	NL
Roufosse et al [11]	F	16	18 ⁿ	ECZ, sc nod	Synovitis	8920	4630	340	IgM 310	90% (CD4)	7 ⁻ 27 ⁻ RO ⁺ 95 ⁺ DR ⁺	Y	Y	IL-4, IL-13 IL-2	Partial 6q 10p del

Bank et al [12]	F	21	12 ⁿ	ECZ, EA	—	9100	3420	15,640	IgM 1250	84% (CD4)	7 ⁻ 27 ⁻ RO ⁺ Y 95 ⁺ DR ⁺	Y	IL-4, IL-13 IL-2	Partial 6q del
	F	47	19 ⁿ	URT	—	2970	2240	478	IgG 1920	16% (CD4)	7 ⁻ 27 ⁻ RO ⁺ Y 95 ⁺ DR ⁺	Y	IL-4, IL-13 IL-2, IFN- γ	NL
	F	38	6	ECZ, sc nodules	LN	8230	5500	113,000	ND	73%	2 ⁺ 5 ⁺ TCR α / β ⁻	ND	IL-4, IFN- γ	ND
	M	70	4	NOD, plaques	Pleuritis, DVT, fever	6000	4100	325,000	ND	66%	2 ⁺ 5 ⁺ 7 ⁻ Y TCR α / β ⁻	Y	IL-4, GM-CSF	ND
	F	46	13	“rash”	Asthma, articul, DVT, fever	6426	8694	NL	ND	95%	2 ⁺ 5 ⁺ 7 ⁻ Y TCR α / β ⁻	ND	ND	ND
Sugimoto et al [23]	F	55	10	PAP, ULC	Pulmon infiltr	13,700	11,500	11,970	—	98% (CD4)	2 ⁺ 5 ⁺ 7 ⁻ Y RO ⁺ TCR α / β ⁻	ND	IL-4, IFN- γ	t(2;14), t(12;14)
Roumier et al [21]	M	20	—	EA, ECZ	LN	9500	10,900	Incr	+	96%	7 ⁻ 27 ⁻ 62L ⁺ Y 25 ⁻ RO ⁺ (biC) DR ⁺ 95 ⁺	Y	IL-4, IL-13 IL-2 IFN- γ , TNF	Trisomy 7
Morgan et al [39]	M	44	13	EA, URT	—	27,500	ND	800	IgM 340	ND	2 ⁺ 7 ⁺ 25 ⁺ 95 ⁺ Y	ND	ND	NL
Vaklavas [30]	M	29	20	PAP	Sinus, oronasal, LN	3300	960	10,853	ND	ND	2 ⁺ 5 ⁺ 7 ⁻ Y	ND	ND	NL
Roufosse > 2007 ⁿ	M	45	3.5	EA	—	3270	3000	8546	ND	ND	2 ⁺ 7 ⁻ 5 ^{hi} 7 ⁻ 27 ⁻ Y RO ⁺ 95 ⁺ 25 ⁺	ND	ND	ND
	M	36	14	EA	Intestinal, articular	5350	5167	<3.5	IgM 400	31%	5 ^{hi} 7 ⁻ 27 ⁻ Y RO ⁺ 95 ⁺ 25 ⁺ DR ⁺	Y	IL-2, IL-4, IL-13	NL
	M	43	2.5	ECZ	—	870	1110	7849	—	9%	5 ^{hi} 7 ⁻ 27 ⁻ Y RO ⁺ 95 ⁺ 25 ⁺ DR ⁺	Y	IL-2, IL-4, IL-13, TNF	NL

(continued on next page)

Table 1 (continued)

Ref: Author	Gender	Age ^a FU		Clinical features		Biologic features			CD3 ⁺ CD4 ⁺ subset				Karyotype	
		(yr)	(y)	Skin ^b	Other ^c	Eosino ^d /μL	Lympho ^e /μL	IgE ^f U/mL	Hyperγglob ^g (mg/dL)	% gated LC ^h	Surface markers ⁱ	Clonality ^j	IL-5 ^k	Other cytokines ^l
	M	25	9	EA, ECZ URT	LN	9600	1680	22	—	0.6%	5 ^{hi} 7 ⁻ 27 ⁻ RO ⁺ 95 ⁺ 25 ⁺	N	Y	IL-2, IL-4, NL IL-13

Abbreviations: FU, follow-up; GM-CSF; granulocyte-macrophage colony-stimulating factor IFN, interferon; IL, interleukin; N, no; ND, not done or not available; NL, normal; TNF, tumor necrosis factor; Y, yes.

^a Age at discovery of eosinophilia or initiation of characteristic symptoms. In a few cases where information on chronology of disease is not available, age at evaluation by authors is given.

^b Type of cutaneous manifestations is abbreviated as follows: EA, episodic angioedema; ECZ, eczema; ERY, erythroderma; NOD, nodular lesions; sc NOD, subcutaneous nodules; PAP, papular dermatitis; ULC, ulcerative lesions; URT, urticaria.

^c Involvement of tissues and organs other than the skin are indicated as follows: —, none; DVT, deep vein thrombosis; LN, lymph node.

^d Highest eosinophil level reported.

^e Normal values for lymphocytes vary, but are roughly between 1340 and 3950/μL.

^f Normal serum IgE levels vary among investigators; in general, below 150 U/mL.

^g Hypergammaglobulinemia is indicated as follows: —, none +, present, but no details available; IgG or IgM indicates the type of immunoglobulin, which is increased in serum, eventually followed by the titre in mg/dL when available.

^h The proportion of CD3⁺CD4⁺ cells is given as the percentage of all lymphocytes in most cases (this includes T cells, B cells, and NK cells); if values are available only as a percentage of a gated lymphocyte subset (eg, on CD4 T cells), this is indicated in the table between parenthesis.

ⁱ Other phenotypic characteristics of the CD3⁺CD4⁺ T cells are indicated when available. The abbreviation for “cluster differentiation” (ie, CD) for the different antigens is not indicated; “CD7” is represented as “7.” RO refers to CD45RO, and DR refers to HLA-DR.

^j T cell clonality is considered present (Y for yes) only when demonstrated using Southern blot or polymerase chain reaction analysis for TCR rearrangement patterns.

^k Increased IL-5 production in patients with CD3⁺CD4⁺ cells is qualified as present (Y for yes) only if this is shown at the single-cell level for the aberrant subset by flow cytometry or immunohistochemistry, or if markedly increased levels are secreted into culture supernatants by stimulated peripheral blood mononuclear cells compared with controls. If low-level IL-5 is detected in culture supernatants of nonstimulated peripheral blood mononuclear cells, this is indicated as “spon.”

^l Production of other cytokines by CD3⁺CD4⁺ cells is indicated when assessed.

^m The long-term follow-up of the patient reported here is found in reference [12] (patient 2).

ⁿ F. Roufosse, personal unpublished observations, 2007.

Table 2
Lymphocytic-hypereosinophilic syndrome associated with T-cell subsets other than CD3-CD4+

Ref.	Author	Gender	Clinical features				Biologic features			Aberrant T-cell subset				
			Age (y)	FU (y)	Skin	Other	Eosino / μ L	Lympho / μ L	IgE U/mL	Phenotype	% gated LC	Clonality	IL-5	Other cytokines
Simon et al [29]	M	44	20y	—	—	Liver, intestine, spleen	4420	3888	12	CD3 ⁺ CD4 ⁺ CD8 ⁺ CD5 ⁺ TCR α / β ⁺ CD95 ⁺	15%	Y	Y	IL-3, IL-2
Kitano et al [28]	M	53	—	—	—	Cardiac thrombus, liver	6270	1870	ND	CD3 ⁺ CD4 ⁺ CD8 ⁺	56% (T cells)	Y	Y	No GM-CSF or IL-3
Simon et al [10]	F	47	5	ERY	—	Lungs	2768	3955	12,050	CD3 ⁺ CD4 ⁺ CD2 ^{lo} CD5 ^{lo}	23.3%	Y	Y	No spon IL-4, IFN- γ
	M	71	1	ERY	—	—	2457	1638	1645	CD3 ⁺ CD4 ⁺ V β 5a ⁺	16%	N	Spon	Spon IL-4, IFN- γ
	M	54	0.2	PAP	—	Spleen	1188	2046	87	CD3 ⁺ CD8 ⁺ CD6 ^{hi}	6.5%	N	Spon	No spon IL-4, IFN- γ
	M	55	4	—	—	spleen, lungs	869	2133	<2	CD2 ^{lo} CD3 ^{lo} CD6 ^{lo} CD7 ^{lo} CD8 ^{hi}	20%	N	—	No spon IL-4
	F	88	13	Poikil.	—	—	5805	810	5	CD3 ⁺ CD4 ⁺ CD7 ⁺ V β 5c ⁺	14.5%	N	Y	Spon IL-4
	M	60	2	ERY	—	—	3180	2438	416	CD3 ⁺ CD4 ⁺ CD25 ⁺	50%	N	Y	Spon IFN- γ , no IL-4
	M	63	8	URT	—	—	1326	867	36	CD3 ⁺ CD4 ⁺ CD8 ⁺	25%	N	Spon	Spon IFN- γ , no IL-4
	M	87	1	PAP	—	—	2323	1314	115	CD2 ^{hi} CD3 ⁺ CD4 ⁺ CD7 ⁺ CD6 ^{lo}	18%	N	—	No spon IL-4, IFN- γ
	F	59	5	ERY	—	—	1332	738	200	CD3 ⁺ CD4 ⁺ CD7 ⁺ and CD3 ⁺ CD4 ⁺ CD8 ⁺	20 and 25%	N	Y	ND
	M	5	3	ERY	—	—	2760	5700	156	CD3 ⁺ CD8 ⁺ CD5 ^{lo} CD7 ^{lo}	22%	Y	—	ND
Vaklavas 2007	M	60	10	—	—	Lungs, LN, kidneys	1150	2640	412	CD3 ⁺ CD8 ⁺ CD5 ^{lo}	ND	Y	ND	ND
	M	62	5.5	ECZ	—	—	720	1120	ND	CD3 ⁺ CD4 ⁺ CD7 ^{lo}	ND	Y	ND	ND
	M	51	4.8	+	—	—	2500	4100	25,360	CD3 ⁺ CD4 ⁺ CD7 ⁺	ND	Y	ND	ND

See footnotes to Table 1.

Persistent hypereosinophilia in patients with this aberrant T-cell subset indicates sustained IL-5 production in vivo, presumably triggered by T-cell stimuli other than antigen major histocompatibility complex. Interestingly, despite complete absence of membrane TCR/CD3, these cells respond to activating signals provided by antigen-presenting cells in vitro, through engagement of CD2 and CD28 costimulatory receptors [18]. Indeed, CD3⁻CD4⁺ cells cultured in presence of mature dendritic cells proliferate, up-regulate membrane CD25 expression, and secrete Th2 cytokines and IL-2, all of which are inhibited if the cocultures are performed in presence of blocking antibodies interfering with CD2 and CD28 engagement. TCR-independent activation of CD3⁻CD4⁺ cells by antigen-presenting cells in vivo may contribute to IL-5 secretion, and ultimately to hypereosinophilia. In addition, combined IL-2 production and expression of the high-affinity receptor for this cytokine by CD3⁻CD4⁺ cells in these conditions, may be involved in chronic cycling of this T-cell subset and emergence of clonality. In this regard, it is of interest that normal CD4 T cells cultured in the presence of anti-CD2 + anti-CD28 antibodies combinations in vitro were shown to display prolonged IL-2-dependent autocrine proliferation, compared with cells cultured in the presence of the more classical anti-CD3 + anti-CD28 antibodies combination [24]. This finding was associated with increased IL-2R α (CD25) and IL-2R β (CD122) mRNA transcription and stability [25,26].

In vitro findings on CD3⁻CD4⁺ cells have led the authors to hypothesize that acquisition of this unusual phenotype is an early event, which exposes these CD4⁺ T cells to alternative TCR-independent stimuli. The downstream signaling pathways may secondarily be implicated in acquisition of the functional behavior that characterizes this lymphocyte subset, including Th2 cytokine production and prolonged clonal expansion.

Molecular characterization of CD3⁻CD4⁺ T cells

To understand better the pathogenesis of L-HES, mechanisms involved in loss of TCR-CD3 complex expression by CD4 T cells have been explored. A marked decrease of CD3 γ chain mRNA was observed in CD3⁻CD4⁺ T cells isolated from HES patients, using Northern blot and microarray analyses [13], contrasting with conserved transcription of the other chains composing the TCR-CD3 complex (TCR α , TCR β , CD3 δ , CD3 ϵ) with the possible exception of CD3 ζ . Successful assembly of the TCR-CD3 complex in the endoplasmic reticulum and subsequent membrane translocation depends on the presence of all TCR and CD3 chains; decreased CD3 γ chain gene expression has been shown to account for low TCR-CD3 expression on in vitro HIV-infected CD4 T cells [27]. Electromobility shift assays were performed to investigate regulatory mechanisms acting on the CD3 γ chain promoter in CD3⁻CD4⁺ cells; nuclear extracts were shown to bind intensely to two NF-AT consensus sequences, NF-AT γ 1 and NF-AT γ 2 [13]. The latter binds NFATc2 containing complexes and exerts negative

regulation on CD3 γ gene transcription, whereas the former may exert positive or negative activity, depending on whether NF-ATc1 plus NF- κ B p50 or NF-ATc2-containing complexes are bound, respectively. Super-shift assays demonstrated binding of large amounts of NF-ATc2 to the NF-AT γ 1 sequence. Inhibitory activity of specific members of the NF-AT family on the CD3 γ gene promoter may contribute to acquisition of this unique T-cell phenotype, but upstream primary events remain entirely unknown.

Lymphocytic hypereosinophilic syndrome associated with T-cell abnormalities other than the CD3 $^-$ CD4 $^+$ phenotype

Besides CD3 $^-$ CD4 $^+$ T cells, other phenotypically aberrant or clonal T-cell subsets have been described in patients fulfilling HES diagnostic criteria (see Table 2) [10,28–30]. For some, however, formal evidence that they produce IL-5 or other eosinophil growth factors has not been provided. In addition, functional and molecular characteristics of these subsets have not been studied as extensively as for CD3 $^-$ CD4 $^+$ cells, with the exception of CD3 $^+$ CD4 $^-$ CD8 $^-$ T cells. Two groups have provided a more detailed analysis of this subset [28,29], showing that the cells express TCR α/β but not TCR γ/δ ; are monoclonal; and produce IL-5, IL-3, IL-2 and possibly granulocyte-macrophage colony-stimulating factor. CD3 $^+$ CD4 $^-$ CD8 $^-$ cells from one patient were shown to display deficient Fas-mediated apoptosis despite normal expression of the Fas-R, CD95 [29]. Reverse transcriptase polymerase chain reaction analysis of Fas-R transcripts demonstrated the existence of two polymerase chain reaction products with different lengths; the shorter splice variant encoded a soluble Fas-R molecule, which interfered with engagement of the membrane-bound receptor, providing a possible explanation for clonal expansion.

Among other T-cell phenotypes associated with HES, CD4 or CD8 subsets with abnormal staining intensities for the lineage-specific T-cell markers CD2, CD5, CD6, and CD7 have been reported [29,30]. In particular, a CD4 T-cell subset with weak or absent membrane CD7 expression seems to be a recurrent abnormality, with five reported cases (see Table 2). Lymphocytes with this phenotype have been observed in affected skin from one HES patient, and stained positively for intracellular IL-5, suggesting a pathogenic role [29]. Clonality was proved by molecular studies in two cases [30], and was highly probable in another given homogenous expression of V β 5c by the CD4 $^+$ CD7 $^-$ subset [10]. The relevance of CD4 $^+$ CD7 $^{\text{dim/-}}$ T cells in HES pathogenesis clearly warrants further investigation.

It is intriguing that the two most frequently encountered aberrant T-cell subsets besides CD3 $^-$ CD4 $^+$ cells in L-HES patients (ie, CD3 $^+$ TCR α/β $^+$ CD4 $^-$ CD8 $^-$ and CD3 $^+$ CD4 $^+$ CD7 $^-$ cells) are observed in the epidermis of healthy individuals [31,32], and are thought to contribute to the “skin-associated immune system.” A modest population of CD4 $^+$ CD7 $^-$ cells is also found in peripheral blood [33] (representing up to 19% of CD4 T cells)

and tends to increase with age. One group has shown that cultured $CD4^+CD7^-$ T cells from healthy subjects produce more IL-4 and less IL-2 than $CD4^+CD7^+$ T cells, after stimulation with anti-CD3 + anti-CD28 antibodies, suggesting that the former are skewed toward a Th2-like profile [34]. Monoclonal $CD3^+TCR^{\alpha/\beta+}CD4^+CD8^-$ and $CD3^+CD4^+CD7^-$ subsets have also been observed in skin from patients with cutaneous T-cell lymphoma [35,36], and high percentages of $CD4^+CD7^-$ cells may be detected in blood of patients with Sézary syndrome [37]. These findings suggest that T cells associated with certain forms of cutaneous T-cell lymphoma may represent the malignant counterpart of T cells that normally contribute to skin defense.

Clinical features and prognosis of lymphocytic hypereosinophilic syndrome

L-HES affects females and males in roughly equivalent proportions, contrasting with the high male/female ratio observed in the overall HES population, which is likely to reflect the overwhelming male predominance of FIP1L1-PDGFR α -associated disease. Age of diagnosis is similar to that of the overall HES population (see Tables 1 and 2).

The clinical profile of patients with $CD3^-CD4^+$ and $CD4^+CD7^-$ T-cell subsets is dominated by cutaneous manifestations, including pruritus, eczema, erythroderma, urticaria, and angioedema. In some cases, the presentation is indistinguishable from that encountered in episodic angioedema with eosinophilia [11,30,38,39], or Gleich's syndrome, a disease characterized by spontaneously remitting episodes of angioedema associated with increased IgM levels. Observed presence of pathogenic $CD3^-CD4^+$ or $CD4^+CD7^-$ T cells together with eosinophils in affected skin from HES patients [10,30] suggests they play a role in cutaneous manifestations. The fact that both populations lack membrane CD7, which is a characteristic of normal epidermal and dermal CD4 T cells [31], may explain preferential homing of pathogenic T cells in the skin, which secondarily attract eosinophils. The respective contributions of these activated T cells versus eosinophils to clinical manifestations remain to be elucidated. Other reported complications associated with $CD3^-CD4^+$ cells include tenosynovitis [11], arthralgia [12], digital arterial occlusion complicated by necrosis [8,19], deep venous thrombosis [12], asthma [12], pulmonary infiltrates [8,23], and eosinophilic pleuritis [12]. Intriguingly, endomyocardial fibrosis or presence of thrombi in cardiac cavities is a rare complication (only one reported case [40]), despite high eosinophil levels.

As for the reported patients with $CD3^+CD4^-CD8^-$ cells (four published cases), skin lesions were the only complication in two [10]: one presented predominant gastrointestinal complications associated with splenic and hepatic eosinophilic infiltrates [29], and one had a thrombus in the left ventricle and hepatomegaly with eosinophilic infiltration of the portal area [28].

Clinical manifestations associated with other less frequent T-cell phenotypes are shown in [Table 2](#).

Biologically, patients with L-HES generally have circulating eosinophil levels well above the defining 1500/ μ L cutoff for HES diagnosis, and absolute lymphocyte counts are normal or slightly increased (see [Tables 1 and 2](#)). In accordance with the type 2 cytokine profile of the aberrant T cells, serum IgE levels are often increased. Polyclonal hypergammaglobulinemia (increased serum IgM or IgG) may be observed but is less common. This contributes to increased erythrocyte sedimentation rates that are observed in some patients, whereas C-reactive protein levels are generally normal or only slightly increased. Features typically associated with myeloproliferative disease, including anemia or thrombocytopenia, presence of circulating immature myelocytes, and increased vitamin B₁₂ levels, are generally absent. Mild lymph node enlargement may be observed, and biopsies show eosinophils and lymphoid hyperplasia [8,20]. Organomegaly is rare, and reflects eosinophilic infiltration. Bone marrow smears contain increased proportions of eosinophil precursors and mature eosinophils.

Short-term prognosis of CD3⁺CD4⁺ T cell-associated HES is clearly better than that of FIP1L1-PDGFR α ⁺ chronic eosinophilic leukemia, given the low incidence of severe or life-threatening end-organ damage and good response to corticosteroid therapy. Long-term prognosis is overshadowed, however, by the possible development of peripheral T-cell lymphoma, many years after HES diagnosis in some cases ([Table 3](#)) [10,11,19,30,41]. Malignant progression has been observed in patients with clonal CD3⁺CD4⁺, CD3⁺CD4⁺CD8⁺, and CD4⁺CD7⁺ subsets, and in some cases the malignant T cells conserve the initial phenotype, suggesting their premalignant nature. The reported duration of the eosinophilic prodrome is variable, and ranges from 3 to 20 years (mean, 7 years). Few details are available on the histology and grading of lymphomas that develop in this setting. A recent article has described progression toward primary cutaneous peripheral T-cell lymphoma, unspecified, in two HES patients, one with CD4⁺CD7⁺ and the other with CD3⁺CD4⁺ T cells [30]. They both presented with papular dermatitis at HES diagnosis, which progressed to more nodular infiltrative lesions when malignancy was detected. The patient with CD3⁺CD4⁺ cells also developed lymphadenopathy and oronasal involvement.

Diagnosis of lymphocytic hypereosinophilic syndrome

Critical analyses for approaching L-HES diagnosis include both peripheral T-cell phenotyping by flow cytometry, and assessment of T-cell clonality by investigating TCR gene rearrangement patterns. Investigations can be performed on peripheral blood samples, because all abnormal T-cell subsets associated with HES reported have been detected in the circulation. Isolated

Table 3
Malignant T-cell lymphoma in patients with initial diagnosis of lymphocytic-hypereosinophilic syndrome

Ref	L-HES characteristics		Duration benign HES (y)	Clinical changes associated with transformation	Characteristics of malignant T-cell lymphoma			
	T-cell phenotype	Clonality			Diagnostic biopsies	Type of lymphoma	Treatment	Outcome
O'Shea et al [19]	CD3 ⁺ CD4 ⁺ CD7 ⁻	ND	7	Modif skin lesions	Skin, lymph node	Diffuse mixed small and large cell	Nitrogen mustard, VCR, PDN, procarbazine	Deceased, <i>Pneumocystis carinii</i> infection
Bagot et al [41]	CD3 ⁺ CD4 ⁺ CD7 ⁻	Y	5	Extension of skin lesions	Skin, lymph node	Pleomorphic, large cell	CYP, PDN, Vindesine, total lymphoid irradiation, PUVA	Rapid recurrence; stable under and dependent on IFN- α
Cogan et al [8] ^a	CD3 ⁺ CD4 ⁺ CD7 ⁻	Y	6	Fever, anorexia, night sweats	Bone marrow, lymph node, liver	Diffuse anaplastic CD30 ⁺	CYP	Deceased, septicemia
Simon et al [29]	CD3 ⁺ CD4 ⁻ CD8 ⁻	Y	20	Increased lymphocytosis, eosinophils	Not specified	Not specified	Not specified	Deceased, small bowel infarction, peritonitis

Simon et al [10]	CD3 ^{lo} CD4 ^{lo} CD7 ⁻	Y	> 7	Increased lymphocytosis, eosinophils	Not specified	Not specified	Not specified	Not specified
	CD3 ^{lo} CD4 ⁺ CD7 ⁻	Y	> 3	Exacerbation skin symptoms	Lymph node	Not specified	Not specified	Not specified
Roufosse et al [11]	CD3 ⁺ CD4 ⁺ CD7 ⁻	Y	6	Subcutaneous nodules, lymphadenopathy, increased lymphocytosis	Lymph node	Diffuse PTCL - pleomorphic, small and medium cell	Fludarabine CHOP-like Allogeneic SCT	Transient partial response No response Complete remission (6 y)
Vaklavas et al [30]	CD3 ⁺ CD4 ⁺ CD7 ^{lo}	Y	3	Infiltrative nodular skin lesions	Skin	Primary cutaneous PTCLu	Multiagent therapy	Partial response
	CD3 ⁺ CD4 ⁺ CD7 ⁻	Y	> 8	Infiltrative nodular skin lesions, lymphadenopathy, oronasal and conjunctival infiltrates	Skin, lymph node	Primary cutaneous PTCLu	Multiagent therapy	Partial response

Abbreviations: CYP, cyclophosphamide; IFN, interferon; PDN, prednisolone; PTCL, peripheral T-cell lymphoma; PUVA, psoralen UVA; VCR, vincristine.

^a The long-term follow-up of the patient reported here is found in reference [12] (patient 2).

demonstration of clonal TCR rearrangements is by no means sufficient to classify a patient as having L-HES, because this may be observed in healthy subjects or may reflect a reactive condition.

Lymphocyte (T cell) phenotyping may be initiated using markers commonly used in routine practice (ie, CD3, CD4, and CD8), given the large predominance of CD3⁺CD4⁺ and CD3⁺CD4⁺CD8⁺ subsets in affected patients. In laboratories unaccustomed to detection of these abnormal populations, quantification of other lymphocytes (staining CD19 for B cells, CD16/CD56 but not CD3 for natural killer cells) may provide indirect evidence for their existence. Indeed, if added percentages of these populations, together with normal CD3⁺, CD4, and CD8 T cells, amounts to less than 90% of gated lymphocytes, this should raise suspicion of an abnormal T-cell subset in an HES patient. Multicolor flow cytometry, staining CD3, CD4, and CD8 together with other lineage-associated T-cell antigens including CD2, CD5, CD6, CD7, CD27, and CD28, and with CD25 is useful for detecting other less frequent phenotypic abnormalities. Besides the two previously mentioned abnormal T-cell subsets, the only recurrent phenotypic abnormality observed in HES patients is low or absent CD7 on CD4⁺ T cells, which should be actively searched. This approach is also useful for confirming the diagnosis of CD3⁺CD4⁺ cells when this subset is small or difficult to distinguish from monocytes (ie, if CD4 expression is slightly down-regulated). Indeed, these cells express CD2 and CD28, and membrane expression of several other T-cell markers is almost systematically altered (CD5^{high}CD7^{neg}CD27^{neg}CD45RO^{pos}) [11,12,21]. Intracellular staining for CD3 ϵ or the TCR α/β framework after permeabilization may further help distinguish this subset from monocytes.

Several pathogenic T-cell subsets have been identified by flow cytometry, by exploring the TCRV β repertoire with a panel of antibodies directed against TCRV β families [10]. An increased percentage of CD4 T cells that stain for a given V β family may arouse suspicion of T-cell clonality. For example, a CD3^{dim}CD4⁺ T-cell subset was shown to stain positively for the V β 6.7 antigen in one patient [10]. Surface staining for V β families is not appropriate for CD3⁺CD4⁺ cells, given the absence of TCR-CD3 on their surface, but intracellular staining after permeabilization is feasible. The authors have attempted to analyze the TCRV β repertoire of CD3⁺CD4⁺ cells from HES patients in this fashion, but have not detected specific V β subsets using a panel of antibodies that covers roughly 60% of families (F. Roufosse, unpublished data, 2000). It is important to note that identification of a T-cell subset belonging to a given V β family does not prove its clonality, which can only be formally demonstrated by molecular analysis of TCR gene rearrangements.

Molecular assessment of T-cell clonality is generally based on Southern blotting for the TCR β chain gene, and polymerase chain reaction analysis for the TCR γ (and recently, TCR β) chains. Most reports of CD3⁺CD4⁺ T-cell populations detected in HES patients show clonal TCR rearrangement

patterns (see Table 1). In several patients, however, investigators have been unable to demonstrate clonality using these techniques, despite high percentages of abnormal cells in peripheral blood [12,20]. In the same line, molecular analyses confirmed T-cell clonality in only 8 of 16 patients with T-cell subsets bearing various phenotypic abnormalities in one cohort [10]. Several explanations are possible, including lack of sensitivity if the population is small; truly nonclonal nature of the studied population; type of chosen primers relative to the rearrangement, deletion, or translocation of the TCR β gene; and incomplete rearrangements. The authors have reported one patient with 16% CD3⁻CD4⁺ cells (among gated CD4⁺ lymphocytes) in whom TCR gene rearrangement studies failed to show clonality on whole blood using polymerase chain reaction analysis for the TCR γ chain gene, but a clonal pattern was observed after in vitro purification of the abnormal population [11].

Given the number of observations and reports showing that clonal CD3⁻CD4⁺ cells produce IL-5 (see Table 1), it is reasonable to classify a new patient in whom this particular T-cell subset is detected as L-HES, even if cytokine production has not been investigated. In contrast, other suspected cases of L-HES on the basis of flow cytometry or analysis of TCR gene rearrangement studies should be characterized extensively to assess a causal relationship between presence of aberrant or clonal T cells and hypereosinophilic disease. To this end, T-cell cytokine production should be investigated by culturing peripheral blood mononuclear cells in the absence and presence of T cell-specific stimulating agents for 48 hours, and measuring cytokines in culture supernatants, with a special focus on IL-5. Optimal stimulation is observed using a phorbol ester, such as phorbol 12-myristate 13-acetate, which bypasses proximal signaling events related to cross-linking of membrane receptors, combined with a calcium ionophore or anti-CD28 antibodies. If increased IL-5 concentrations are measured in peripheral blood mononuclear cells supernatants, experiments can be repeated after depletion of specific T-cell subsets (eg, using anti-CD3 or anti-CD4 antibodies) for refining assessment of the type of T cell responsible for IL-5 overproduction [12,20]. If a T-cell subset with a phenotypic abnormality is detected (eg, CD3⁻CD4⁺, CD3⁺CD4⁻CD8⁻, or CD4⁺CD7^{dim/-}), assessment of cytokine production at the single-cell level is possible using flow cytometry, by staining intracellular cytokines together with relevant surface markers, after a short course of in vitro T-cell stimulation with the previously mentioned agents [10,11]. Although these techniques are not standardized (eg, investigators report using different concentrations of cells and reagents, variable culture durations, and so forth), the authors generally run cultures of peripheral blood mononuclear cells from HES patients and from healthy subjects in parallel, and results are generally straightforward, with markedly increased IL-5 levels in supernatants for patients with L-HES.

In routine practice, diagnosis of L-HES is laborious, and may be stalled by the inability to interpret results of flow cytometry or TCR rearrangement patterns, and lack of equipment or expertise to culture T cells and assess

cytokine production. Identification of L-HES patients would be facilitated if a straightforward, reproducible, and unique diagnostic test were available. One study has identified increased serum thymus and activation-regulated chemokine (TARC) as a potential biomarker for this variant [42]. Serum TARC levels are indeed over 100-fold higher in patients with L-HES (mean, 56,564 pg/mL; range, 15,459–161,032; $N = 10$) compared with HES patients with no evidence for an underlying T-cell disorder (mean, 296 pg/mL; range, 91–892; $N = 8$), whose levels were similar to healthy controls (mean, 265 pg/mL; range, 66–625; $N = 38$) (F. Roufosse, unpublished data, 2007). In vitro studies have shown that the high-level TARC production observed when $CD3^+CD4^+$ cells are cultured in presence of dendritic cells is inhibited when engagement of the IL-4R is hindered with blocking antibodies [43], indicating that the pathogenic T cells are directly responsible for TARC synthesis by other cell types, through production of IL-4 or IL-13. Similarly, IL-4 has been shown to increase TARC production by human bronchial epithelial cells [44] and murine Langerhans cells [45] in vitro, and serum TARC levels seem to correlate with disease activity in atopic individuals [46], albeit much less dramatically than in L-HES patients. Increased serum TARC may truly be the hallmark of diseases mediated by activated Th2 cells. The fact that this biomarker can be measured in serum (which can easily be stored and shipped) using a commercially available ELISA kit (possibility of standardization, reproducibility) makes it a very appealing diagnostic test. Further studies are required, however, to assess the specificity of this candidate biomarker for L-HES with regard to other patients fulfilling HES diagnostic criteria, and to determine discriminative cutoff values for this variant. To this end, serum TARC should be compared between larger cohorts of L-HES and other HES patients, and healthy subjects and patients with other conditions associated with hypereosinophilia, including atopy, drug allergy, parasitosis, Churg-Strauss syndrome, and organ-specific eosinophil-mediated disorders (eg, eosinophilic gastroenteritis and eosinophilic pneumonia).

Follow-up of patients with lymphocytic hypereosinophilic syndrome

Given the increased risk of developing peripheral T-cell lymphoma, patients with L-HES should be monitored on a regular basis to detect this complication as early as possible. It must be underlined, however, that malignant progression has been observed only in a proportion of patients, whereas most pursue a benign course, with reassuring long-term follow-up in some published cases [11,23,39]. Malignant progression has been observed in association with $CD3^{+/dim}CD4^+$, $CD3^+CD4^-CD8^-$, and $CD4^+CD7^{dim/-}$ subsets several years after detection of monoclonal T cells (see Table 3). This raises the difficult question of when clonal T cells bearing phenotypic abnormalities should be considered malignant. In most reported cases, diagnosis of lymphoma is made when a biopsy (lymph node, skin)

reveals the presence of morphologically abnormal lymphocytes. Clinical manifestations associated with malignant transformation include appearance of enlarged lymph nodes [30,47] and progression of skin lesions from papular dermatitis to infiltrative nodules [30]. Hematologically, rapid increases of lymphocytosis (essentially involving the aberrant T cells) and eosinophilia [10], and increased expression of CD25 by CD3⁺CD4⁺ cells [47], have been observed.

Another critical question regarding L-HES and malignancy is whether patients who are more likely to develop lymphoma can be singled out on the basis of specific disease characteristics (have any predictive markers been identified). Repeated karyotypes on CD3⁺CD4⁺ cells during a period of several years in one patient who eventually developed lymphoma have shown that the nonmalignant CD3⁺ T cells present at diagnosis contained two distinct partial 6q deletions (6qdel[q13-q22] and 6qdel[q11-q23]), whereas their malignant counterpart 6 years later presented only the short 6qdel(q13-q22) [48]. The fact that this deletion involves chromosomal loci whose absence may be associated with other malignancies (including T-cell and B-cell disorders and solid tumors) strongly suggests that it harbors a tumor suppressor gene, whose nature remains unknown. Parallel investigations in another patient with L-HES have revealed a similar 6q deletion that has remained stable under corticosteroid treatment for 10 years, however, with no evidence of malignancy [48]. Other cytogenetic abnormalities have been reported in L-HES patients, including -16,+der(16)t(16q22;?) in CD3⁺CD4⁺CD8⁺ cells [28] and trisomy 7 in CD3⁺CD4⁺ cells [21], but were not associated with malignant progression at time of reporting. Peripheral blood samples from L-HES patients should systematically be subjected to karyotyping, to characterize better the prognostic significance of specific abnormalities, including partial 6q deletions. The fact that CD3⁺CD4⁺, CD3⁺CD4⁺CD8⁺, and probably CD4⁺CD7⁺ T-cell subsets respond poorly to classical mitogens, such as phytohemagglutinin [19,20,28,34], should be taken into account for interpreting cytogenetic studies performed on cells in metaphase. Indeed, normal T cells proliferate preferentially in these conditions, and the mitoses obtained for karyotyping are not representative of pathogenic T cells. For patients with L-HES, karyotypes should be performed using T-cell growth factors, which induce cell-cycling independently of TCR-CD3-linked signaling, such as IL-2, and eventually IL-7 [49].

Practically, tentative recommendations for follow-up of L-HES patients can be made on the basis of longitudinal observations on a handful of patients, from diagnosis of HES to development of lymphoma. Lymphocytosis should be controlled every 3 to 4 months, and absolute levels and proportions of aberrant T cells should be monitored at least twice a year by flow cytometry. Karyotypes should be performed yearly, with appropriate T-cell mitogens. Appearance of enlarged lymph nodes or modification of skin lesions should prompt biopsies for histologic assessment. Although no data support use of fluorodeoxyglucose positron emission tomography for

follow-up of patients with premalignant T cells, the authors perform this yearly in selected patients with CD3⁺CD4⁺ cells.

Therapeutic considerations

The decision to treat a patient with L-HES depends on a critical assessment of clinical disease severity, in light of treatment-related toxicity. Some patients with T-cell clones have very limited cutaneous manifestations, and it may be reasonable to watch and wait despite high eosinophil levels. For instance, one elderly female patient with clonal CD3⁺CD4⁺ T cells, eosinophil levels around 3000/ μ L, and intermittent episodes of urticaria has not received treatment for 18 years, and has remained stable over time [11]. In others, although clinical manifestations are not life-threatening, they are nonetheless intolerable; typically, extensive pruritic dermatitis requires some form of systemic therapy. In rare instances, preoccupying target-organ involvement represents another indication for treatment. Ideally, therapeutic strategies should target both eosinophils, which are directly involved in end-organ damage and dysfunction, and aberrant T cells, which not only promote hypereosinophilia, but also proliferate clonally and produce mediators other than IL-5, which may contribute partially to disease manifestations. Specific T cell-oriented aims include abrogating production of eosinophilopoietic cytokines or interfering with their effects, and controlling expansion of these cells in hopes of preventing malignant transformation.

Corticosteroids

Corticosteroids (CS) represent first-line therapy for L-HES. Although the response rate in this population remains to be assessed on a well-characterized patient cohort, available data in the literature indicate that satisfactory responses are observed in an important proportion of patients. Apoptosis-inducing effects of CS on eosinophils are well-known, and they also modulate T-cell functions by inhibiting production of type 2 cytokines and IL-2, and by reducing CD25 expression, thereby interfering with clonal IL-2-dependent expansion in vitro [50–52]. In vivo, although CS are very successful in decreasing eosinophil levels in a significant proportion of patients with L-HES, and in controlling clinical manifestations, effects on pathogenic T cells are variable. In most cases, the proportion of CD3⁺CD4⁺ cells is not affected by CS; however, a significant decline of this population has been observed in two patients [11,20].

Practically, it is reasonable to initiate therapy with 20 to 40 mg prednisolone per day, depending on clinical manifestations, followed by progressive tapering so that eosinophil levels and clinical manifestations are controlled using the lowest possible dose. Given the numerous side effects of long-term CS use, therapeutic strategies should aim to minimize overall CS exposure by introducing CS-sparing agents whenever the dose required to control

disease is considered unacceptable. Most of the second-line molecules used for treating the overall HES patient population have been administered to patients with L-HES, with variable success. Compounds used for CS-resistant HES patients and for CS-sparing purposes are the same, and include cytotoxic agents (hydroxyurea); immunomodulatory molecules (IFN- α); tyrosine kinase inhibitors (imatinib mesylate); and monoclonal antibodies (alemtuzumab, anti-IL-5). Allogeneic stem cell transplantation is reserved for patients who develop T-cell malignancy.

Cytotoxic agents

There is currently no evidence indicating that hydroxyurea could be useful for treating lymphoproliferative disorders in general, and its effects on pathogenic T cells isolated from L-HES patients (ie, on IL-5-production and clonal expansion) have never been assessed in vitro. Administration of this agent to one patient with CD3⁺CD4⁺ cells was followed by clinical improvement and normalization of eosinophil levels, presumably through a central negative effect on cytokine-driven eosinophil expansion, but the aberrant T-cell clone remained unaffected [23]. Similarly, one group has recently reported use of other cytotoxic compounds (methotrexate and cyclophosphamide) in two patients with clonal T cells, showing successful control of clinical manifestations, but lack of effect on the T-cell clones [30].

Immunomodulatory agents

IFN- α is an interesting alternative for management of patients with L-HES, because it has been shown to antagonize Th2 responses in general, both in vitro and in vivo [53,54], and more specifically to inhibit proliferation of and decrease IL-5 production by CD3⁺CD4⁺ cells isolated from patients with L-HES in a dose-dependent manner in vitro [17,55]. IFN- α also has central inhibitory effects on eosinophil colony formation [56]. Published case reports have shown that treatment with IFN- α was followed by partial regression of pathogenic CD3⁺CD4⁺ T cells in two patients [8,10], and the authors have observed complete disappearance of CD3⁺CD4⁺ cells in one patient treated with combined CS and IFN- α (F. Roufosse, unpublished data, 2004). IFN- α also prolongs survival of clonal CD3⁺CD4⁺ cells in vitro by inhibiting spontaneous apoptosis [17], however, and may provide these cells with a selective advantage. Given the malignant potential of aberrant T cells associated with L-HES, it may be reasonable to avoid IFN- α as monotherapy in this setting, and rather use it in association with a therapeutic agent displaying proapoptotic activity on the clonal T-cell population, such as CS (L. Schaudere, unpublished data).

The dose of IFN- α required to control eosinophilia in the overall HES population is variable, but is generally between 7 and 14 million units per week [57]. There are no published data on efficient doses for patients with L-HES. Therapy should be initiated at low doses, and increased progressively

to minimize the debilitating flulike symptoms, which tend to decrease over time. Effects of IFN- α on eosinophil levels and associated symptoms are delayed, meaning that adjustment of optimal dosing may take months.

Tyrosine kinase inhibitors

Imatinib mesylate, the tyrosine kinase inhibitor that targets abl, PDGFRA, PDGFRB, and c-kit, has recently become extremely popular in HES management, given its dramatic effects on cells expressing the FIP1-L1-PDGFR fusion protein [58]. This agent has been administered briefly to several patients with L-HES, none of which has responded in terms of clinical manifestations or eosinophil levels [30,40,59]. To date, studies on aberrant T cells implicated in L-HES pathogenesis have not revealed abnormal tyrosine kinase activity that justifies a clinical trial with imatinib mesylate or other tyrosine kinase inhibitors in this setting.

Monoclonal antibodies

An interesting therapeutic target in patients with L-HES is the CD52 antigen, which is expressed both on T cells and eosinophils. In a recent report, alemtuzumab, a monoclonal anti-CD52 antibody, was shown to be effective treatment for a patient with a CD3⁺CD4⁺ T-cell subset, inducing rapid normalization of eosinophil levels and clinical remission [40]. Effects on the aberrant T cells were not reported. After an initial treatment period with 30 mg subcutaneous alemtuzumab per week, the interval was increased to every 3 weeks with sustained remission. Some patients with Sézary syndrome have developed cardiac dysfunction during treatment, leading authors to suggest possible treatment-related toxicity [60]. This could be a subject of concern for patients with HES, although the patient reported here actually improved cardiac function with treatment.

Monoclonal anti-IL-5 mAbs target eosinophils by interfering with ligation of IL-5 to the α -chain of the IL-5R on their surface. Several open-label studies evaluating effects of intravenous anti-IL-5 in HES patients showed a rapid decline of blood eosinophil counts shortly after administration, associated with improvement of a range of clinical manifestations, allowing CS tapering in some cases [61–63]. Eosinophil depletion and clinical benefit in response to 750 mg intravenous mepolizumab, the anti-IL-5 mAb produced by GlaxoSmithKline, can be surprisingly long-lasting, and in-between dose intervals may be several months long [62]. Anti-IL-5 treatment is expected to be beneficial for patients with L-HES in terms of eosinophil-mediated clinical manifestations, because eosinophil expansion is clearly driven by exogenous (T cell) production of IL-5, but is unlikely to affect pathogenic T cells. As of yet, patients responding to anti-IL-5 treatment in published reports did not have clear-cut T-cell-mediated HES. Efficacy of mepolizumab as a CS-sparing agent in FIP1L1-PDGFR α ⁺ HES patients has very recently been evaluated, however, in

the setting of a randomized, double-blind, placebo-controlled clinical trial (manuscript submitted for publication). It is likely that detailed analysis of patient characteristics and study results will disclose whether the L-HES patient subgroup responds to this strategy. Further analyses within the open-label extension of this clinical trial will provide information on long-term safety and tolerance of anti-IL-5 therapy and associated eosinophil depletion.

Strategies warranting future investigation

Future in vitro investigations could explore effects of innovative agents or strategies on pathogenic T cells from patients with L-HES, including cyclosporine A, anti-IL-2R- α mAb, and extracorporeal photochemotherapy. In patients with CD3⁺CD4⁺ cells, cyclosporine A could interfere with nuclear accumulation of NF-ATc2, which is thought to contribute to decreased transcription of CD3 γ chain gene and enhanced transcription of Th2 cytokine genes. Furthermore, in vitro studies have shown that IL-2–IL-2R- α interactions are critical for survival and proliferation of CD3⁺CD4⁺ cells, and for production of Th2 cytokines [18], providing rationale for both cyclosporine A and anti-IL-2R- α mAb. As for extracorporeal photochemotherapy, induction of T-cell apoptosis and modulation of cytokine profiles in favor of Th1 responses account for suppressive effects on pathogenic T cells associated with cutaneous T-cell lymphoma, atopic dermatitis, and graft-versus-host disease [64–66]. Because pathogenic T-cell clones in HES are found in peripheral blood, they could represent an ideal target for extracorporeal irradiation. Interestingly, one patient with circulating CD3⁺CD4⁺ T cells and skin lesions harboring T cells with a clonal TCR rearrangement pattern was treated by psoralen plus UVA therapy, and regression of the pathogenic T-cell subset was observed in peripheral blood [12], suggesting that the skin may be an important source of these cells.

Management of T-cell lymphoma in the setting of lymphocytic hypereosinophilic syndrome

Once full-blown peripheral T-cell lymphoma has developed in patients initially diagnosed with L-HES, eradication of malignant T cells is not easily achieved using classical chemotherapeutic regimens [12,30]. The purine nucleoside analogue fludarabine may be effective for controlling indolent lymphoid malignancies [67,68] (eg, Sézary syndrome, peripheral T-cell lymphoma) and potently suppresses CD4⁺ T cells, making it an interesting candidate for treating malignant CD3⁺CD4⁺ or CD4⁺CD7⁺ T-cell lymphoma. The authors have administered fludarabine to one patient with a CD3⁺CD4⁺ T-cell clone, who experienced temporary benefit in terms of eosinophil levels and cutaneous symptoms (pruritis, eczema). The authors observed very partial regression of the T-cell clone, however, which increased shortly after the sixth cycle of treatment (F. Roufosse, unpublished

observation, 1999). She was also resistant to standard CHOP-like chemotherapy. Finally, intensification of chemotherapy followed by transplantation of allogeneic stem cells from an HLA-matched sibling successfully and durably eradicated the malignant CD3⁺CD4⁺ T-cell clone, and she is still in complete remission more than 6 years after the procedure.

Summary

The discovery that IL-5-producing T cells are directly involved in hyper-eosinophilia in a subset of HES patients has been followed by extensive functional characterization of the pathogenic T cells, especially CD3⁺CD4⁺ cells, and long-term observation of such patients. These studies have somewhat modified management of this disorder. For example, the former belief that increased serum IgE is associated with a benign disease course has been replaced by concern that patients with phenotypically aberrant T-cell clones may eventually develop peripheral T-cell lymphoma; patient follow-up has been adapted to detect this complication. Although many agree that therapeutic strategies should target T cells and eosinophils, the practical implications remain unclear. The authors are confident that systematic recording of patient responses to currently available therapeutic agents, and identification of novel therapeutic targets through investigation of primary molecular defects underlying L-HES, will result in improved treatment and outcome of this HES variant.

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