

High serum thymus and activation-regulated chemokine levels in the lymphocytic variant of the hypereosinophilic syndrome

Aurore de Lavareille, BSc,^{a,*} Florence Roufosse, MD,^{a,b,*} Peter Schmid-Grendelmeier, MD,^c Anne-Sophie Roumier, MD,^d L. Schandené, PhD,^a Elie Cogan, MD,^b Hans-Uwe Simon, MD,^e and Michel Goldman, MD^a Brussels, Belgium, Davos-Platz and Bern, Switzerland, and Lille, France

The idiopathic hypereosinophilic syndrome is associated with expansion of an IL-5-producing T-cell subset in a subgroup of patients. Identification of such patients is critical to adequate management because there is some evidence that they present an increased risk for development of T-cell lymphoma. Although the T_H2-like cells often bear an aberrant surface phenotype and can readily be detected with flow cytometry, we now show that lymphocyte phenotyping might be normal in some cases. In contrast, serum thymus and activation-regulated chemokine levels are consistently increased in such patients compared with others with persistent idiopathic hypereosinophilia and could therefore represent a useful diagnostic tool. (*J Allergy Clin Immunol* 2002;110:476-9.)

Key words: Thymus and activation-regulated chemokine, hypereosinophilic syndrome, IL-5, T-cell lymphoma

Idiopathic hypereosinophilic syndrome (IHES) is a heterogeneous group of disorders characterized by persistent hypereosinophilia of unknown origin.¹ Investigation of circulating lymphocytes in this syndrome has disclosed that IL-5-producing T-cell subsets are detectable in a subgroup of patients.^{2,3} Although the existence of this subgroup has not yet been widely acknowledged, the strikingly homogenous clinical and biologic profile of such patients² has led us to define the lymphocytic variant (LV) of the IHES as a distinct entity in which eosinophilia is likely to be caused by overproduction of IL-5 by a T_H2-like lymphocyte subset.

Abbreviations used

IHES: Idiopathic hypereosinophilic syndrome
LV: Lymphocytic variant
TARC: Thymus and activation-regulated chemokine

Early identification of an underlying T-cell disorder in some patients with IHES has important therapeutic and prognostic implications. Therapeutic strategies directed against T cells would advantageously replace current recommendations. Moreover, the reported occurrence of peripheral T-cell lymphoma in some patients with the LV of IHES²⁻⁴ suggests the premalignant nature of this disorder. We sought to identify the most useful diagnostic tools for distinguishing patients with the LV from others in the setting of IHES.

METHODS

Patients

Patients with IHES fulfilled the diagnostic criteria initially proposed by Chusid et al¹ in 1975. In addition to those reported in our previous series² (patients 1-9), we recruited 3 additional patients (patients 10-12) and included 5 patients provided by H.-U. Simon (patients 13-17; patients 13-15 correspond to patients 4, 14, and 9, respectively, in the study by Simon et al³). Sera from hypereosinophilic patients with allergic disorders (dermatitis, n = 4; asthma, n = 5; or both, n = 5; eosinophilia, 390-1200/mm³) and parasitosis (strongyloidosis, n = 2; filariasis, n = 1; and toxocarosis, n = 1; eosinophilia, 980-1681/mm³) were provided by P. Schmid-Grendelmeier and were all free of systemic glucocorticoid therapy at the time of sampling. Sera from healthy donors (n = 19) with normal eosinophil levels (<350/mm³) were obtained after informed consent.

Investigation of T cells in patients with IHES

Methods used for the demonstration of increased IL-5 production by T cells from patients 1 to 12 have been published elsewhere (Table I).² For patients 13 to 17, IL-5 levels were measured in 24-hour culture supernatants of unstimulated PBMCs (10⁷/mL). Flow cytometric identification of lymphocyte subpopulations in peripheral blood and assessment of T-cell clonality by means of Southern blotting and PCR analysis for T-cell receptor β/γ chains were performed as previously described.^{2,3} Flow cytometric analysis of the V β repertoire with a panel of mAbs was used in some cases.³

From the Departments of ^aImmunology and ^bInternal Medicine, Hôpital Erasme, ULB, Brussels; ^cSwiss Institute of Allergy and Asthma Research (SIAF), Davos-Platz; ^dthe Department of Immunology, Centre Hospitalier Régional Universitaire, and Unité INSERM 547, Institut Pasteur de Lille, Lille; and ^ethe Department of Pharmacology, University of Bern, Bern.

*A. de Lavareille and F. Roufosse contributed equally to this work.

Supported by the Fonds National de la Recherche Scientifique and the Télévie Programme (Belgium), as well as the Swiss National Science Foundation (grant No. 31-58916.99) and Stiftung für Krebsbekämpfung (Zürich).

Received for publication May 6, 2002; revised June 5, 2002; accepted for publication June 5, 2002.

Reprint requests: Michel Goldman, Department of Immunology, Hôpital Erasme, 808 Route de Lennik, 1070 Brussels, Belgium.

© 2002 Mosby, Inc. All rights reserved.

0091-6749/2002 \$35.00 + 0 1/83/127003

doi:10.1067/mai.2002.127003

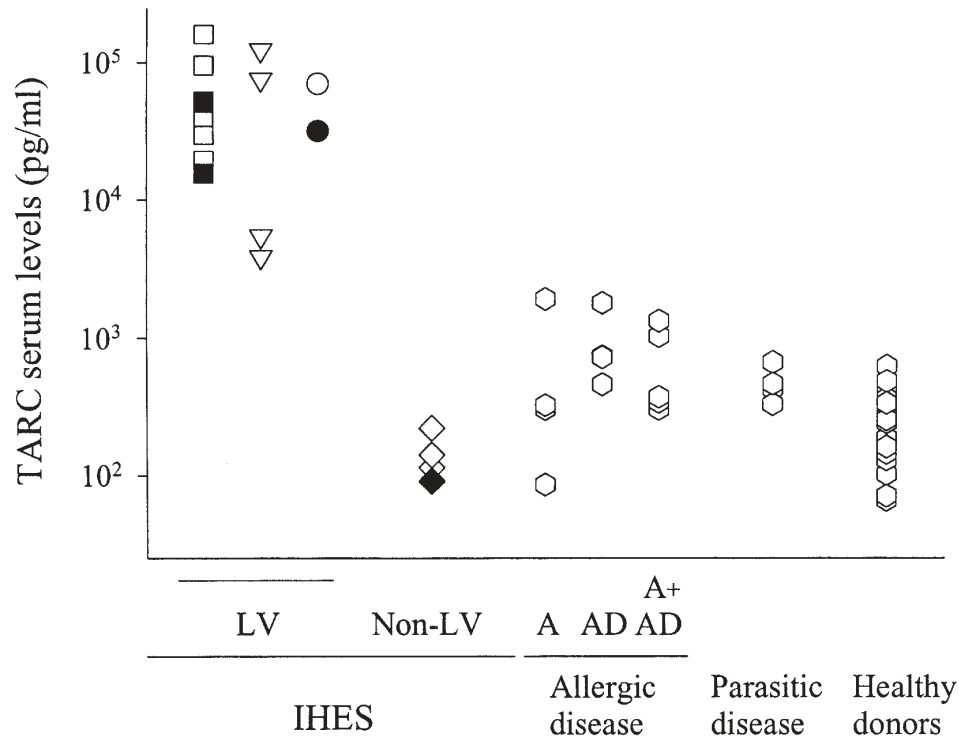


FIG 1. Increased serum TARC levels in patients with the LV of IHES. Serum TARC was measured in patients with IHES, patients with known T_H2-mediated immune responses (allergic and parasitic disease), and healthy control subjects. Patients with allergy (atopic dermatitis [AD], n = 4; asthma [A], n = 5; or both [A+AD], n = 5) and with parasitosis (n = 4) were free of systemic glucocorticoid therapy at the time of sampling, as were those with IHES (open symbols), except for patients 6, 11, 14, and 15 (filled symbols). Among patients with IHES, TARC levels were increased in serum from all patients with the LV compared with those in whom no underlying T-cell disorder was detected (diamonds), whether the former presented an aberrant CD3-CD4⁺ lymphocyte subset (squares), other phenotypically aberrant T cells (circles), or evidence for increased IL-5 production by T cells despite normal lymphocyte phenotyping (triangles).

Measurement of serum thymus and activation-regulated chemokine levels

Serum thymus and activation-regulated chemokine (TARC) levels were determined by using commercially available ELISA kits from R&D Systems Europe. The minimum detectable concentration of TARC is 7 pg/mL.

RESULTS

Identification of patients with the LV of IHES

Patients with IHES were first assessed for evidence of the LV by investigating IL-5 production by circulating T cells. As shown in Table I, increased IL-5 production was observed in 13 of 17 patients. IL-5 hyperproduction was spontaneous for 5 patients (patients 13-17), whereas stimulation with phorbol myristate acetate plus anti-CD28 mAb was needed for patients 1 to 5 and 10 to 12. An aberrant T-cell subset was identified by means of flow cytometry in 9 patients only, including 7 patients with CD3-CD4⁺ or CD3^{low}CD4⁺ cells. T-cell clonality was formally demonstrated in 7 of the 9 patients with phenotypically aberrant T cells and in 2 additional patients with normal phenotyping. Clonality of aberrant T cells was suspected in 2 other patients on the basis of their expres-

sion of a specific V β antigen. Thus among 13 patients with IL-5 overproduction, 2 had normal lymphocyte phenotyping and negative assessment for T-cell clonality.

Increased TARC levels in serum of patients with the LV

Among patients with IHES, serum TARC levels were up to 100-fold higher in those whose PBMCs displayed high IL-5 production compared with those with no evidence for an underlying T-cell disorder and healthy donors (Fig 1), whether they presented clonal CD3-CD4⁺ or CD3^{low}CD4⁺ cells, other phenotypically aberrant T-cell clones, or normal lymphocyte phenotyping.

DISCUSSION

Considering increased IL-5 production by circulating lymphocytes as a defining feature of the LV, 17 patients with IHES were assessed for evidence of this variant. Because measurement of serum IL-5 levels is not reliable in this regard,² we studied production of this cytokine in vitro by PBMCs and observed increased IL-5 levels in 13 patients (Table I). Indeed, affiliated hospitals preferentially referred patients with IHES to our center when they

TABLE I. Classification of patients with IHES on the basis of analysis of circulating T cells

Patient no.	Age (y)	Sex	Glucocorticoids (mg/d)	Lymphocyte count (/mm ³)	Eosinophil count (/mm ³)	IL-5 levels (pg/mL)		Phenotype of abnormal T cells	T-cell receptor analysis			Classification/code
						Spont	PBMC stim		PCR (γ)	SB (β)	FC	
1	21	F	No	4630	8923	<10	70,900 ↑	CD3-CD4 ⁺ (87%)†	+	NT	NT	LV/□
2	18	F	No	3400	9070	<10	63,000 ↑	CD3-CD4 ⁺ (44%)†	+	+	NT	LV/□
3	30	M	No	1484	6120	<10	8700 ↑	CD3-CD4 ⁺ (54%)†	NT	+	NT	LV/□
4	58	F	No	2340	3270	<10	27,750 ↑	CD3-CD4 ⁺ (10.2%)†	+	NT	NT	LV/□
5	30	F	No*	1790	4840	<10	3146 ↑	No	NT	+	NT	LV/▽
6	36	F	mPDN 32	1320	3990	<10	521 normal	No	–	–	NT	Non-LV
7	28	M	No	1910	9150	<10	175 normal	No	–	–	NT	Non-LV
8	65	M	No	2570	13,107	<10	313 normal	No	–	–	NT	Non-LV
9	34	M	No	2660	11,700	<10	92 normal	No	–	–	NT	Non-LV
10	35	M	Unknown	15,200	3920	<10	20,400 ↑	CD3-CD4 ⁺ (84%)†	+	NT	NT	LV/□
11	43	M	PDN 20	5170	841	<10	11,400 ↑	CD3-CD4 ⁺ (29%)†	+	NT	NT	LV/■
12	28	F	No	2910	7425	<10	112,500 ↑	No	+	NT	NT	LV/▽
13	71	M	No	1638	2457	46	NT ↑	CD3 ⁺ CD4 ⁺ CD25 ⁺ (16%)†	NT	NT	Vβ5a	LV/○
14	55	M	PDN 50	824	5047	131	NT ↑	CD3 ^{low} CD4 ⁺ (32.9%)†	+	+	NT	LV/■
15	88	F	PDN 20	810	5805	37	NT ↑	CD3 ⁺ CD4 ⁺ CD7 [–] (14.5%)†	NT	NT	Vβ5c	LV/●
16	57	F	No	2040	9180	125	NT ↑	No	–	NT	NT	LV/▽
17	73	F	No	2187	7687	291	NT ↑	No	–	NT	NT	LV/▽

For LV, the presence of CD3-CD4⁺ cells (*squares*), other aberrant phenotypes (*circles*), or normal lymphocyte phenotyping (*triangles*) is shown. Filled symbols represent patients treated with glucocorticoid therapy at time of sampling.

(m)PDN, (Methyl)prednisolone (*interrupted 1 week before sampling); PBMC stim, IL-5 measured by means of ELISA in supernatants of PBMCs (10⁶/mL) stimulated with phorbol myristate acetate (1 ng/mL) and anti-CD28 mAb (1 μg/mL) for 48 hours (healthy control subjects, 533 pg/mL [SD, ±509 pg/mL]);

Spont, IL-5 measured by means of ELISA in supernatants of PBMCs (10⁷/mL) after 24 hours (healthy control subjects, <5 pg/mL); NT, not tested; SB, Southern blotting (β-chain gene); FC, flow cytometry (clonality suggested by expression of a unique Vβ chain).

†Percentage among total lymphocytes is given in parentheses.

presented features suggestive of the LV. According to a recent study, overproduction of IL-5 can be anticipated in approximately one fourth of patients with chronic idiopathic hypereosinophilia.³

Because the IL-5-producing T cells involved in the pathogenesis of the LV of IHES display aberrant surface phenotypes (eg, CD3-CD4⁺ or CD3⁺CD4-CD8[–]) in all cases reported thus far^{2,3,5-7} and are frequently shown to be monoclonal, investigation of phenotype and clonality of circulating T cells is an important step in detection of this variant. Among the 13 patients with demonstrated IL-5 overproduction in this series, a clonal T-cell subset with an aberrant phenotype was identified in 7 patients, and isolated phenotypic abnormalities (n = 2) or T-cell clonality (n = 2) were detected in 4 additional patients (Table I). Combined flow cytometry and analysis of T-cell receptor rearrangement patterns are not fully reliable for identification of patients with the LV of IHES because both were normal in 2 patients whose PBMCs produced increased IL-5 levels in vitro.

TARC is a CC chemokine thought to be involved in T_H2-mediated immune responses, given its ability to induce selective migration of CD4⁺CD45RO⁺ lymphocytes producing type 2 cytokines.⁸ We measured TARC levels in serum from all 17 patients with IHES and compared results with those of healthy control subjects and patients with allergic or parasitic disease. Our observation that serum TARC levels are slightly increased in patients with atopy and parasitosis (Fig 1 and not shown),

both established T_H2-mediated diseases, is in keeping with a recent study on patients with atopic dermatitis.⁹ Among patients with IHES, serum TARC levels were dramatically increased only in those with the LV, although they did not correlate with the number of circulating clonal cells or eosinophils (data not shown). Interestingly, current glucocorticoid administration did not hinder identification of 3 patients with the LV of IHES in this series, despite known inhibitory effects on TARC production in vivo⁹ and in vitro.¹⁰

The role of TARC in positive amplification loops involving T_H2 cells has been suggested by one study in vitro showing its induction in bronchial epithelial cells in response to IL-4 and IL-13.¹⁰ In favor of a T_H2-specific chemokine-cytokine network operating in the LV of IHES, we have previously shown that CD3-CD4⁺ cells from such patients also produce IL-4 and IL-13 and that inhibition of IL-4 receptor engagement during CD3-CD4⁺/dendritic cell cocultures is followed by an important decrease of TARC secretion into supernatants.¹¹

In conclusion, our data suggest that measurement of serum TARC levels could represent a precious and discriminative diagnostic tool for clinicians dealing with patients with IHES because increased values are observed only in patients with the LV. Preferably, patients should be free of glucocorticoid treatment at the time of sampling, although our study suggests that this precaution is not critical.

We thank P. Stordeur for providing serum from healthy donors.

REFERENCES

1. Chusid MJ, Dale DC, West BC, Wolff SM. The hypereosinophilic syndrome: analysis of fourteen cases with review of the literature. *Medicine (Baltimore)* 1975;54:1-27.
2. Roufosse F, Schandené L, Sibille C, Willard-Gallo K, Kennes B, Efira A, et al. Clonal Th2 lymphocytes in patients with the idiopathic hypereosinophilic syndrome. *Br J Haematol* 2000;10:540-8.
3. Simon HU, Plotz SG, Dummer R, Blaser K. Abnormal clones of T cells producing interleukin-5 in idiopathic eosinophilia. *N Engl J Med* 1999;341:1112-20.
4. O'Shea JJ, Jaffe ES, Lane HC, MacDermott RP, Fauci AS. Peripheral T cell lymphoma presenting as hypereosinophilia with vasculitis. Clinical, pathologic, and immunologic features. *Am J Med* 1987;82:539-45.
5. Brugnoni D, Airo P, Rossi G, Bettinardi A, Simon HU, Garza L, et al. A case of hypereosinophilic syndrome is associated with the expansion of a CD3-CD4+ T-cell population able to secrete large amounts of interleukin-5. *Blood* 1996;87:1416-22.
6. Cogan E, Schandené L, Crusiaux A, Cochaux P, Velu T, Goldman M. Brief report: clonal proliferation of type 2 helper T cells in a man with the hypereosinophilic syndrome. *N Engl J Med* 1994;330:535-8.
7. Kitano K, Ichikawa N, Mahbub B, Ueno M, Ito T, Shimodaira S, et al. Eosinophilia associated with proliferation of CD(3+)4-(8-) alpha beta+ T cells with chromosome 16 anomalies. *Br J Haematol* 1996;92:315-7.
8. Imai T, Nagira M, Takagi S, Kakizaki M, Nishimura M, Wang J, et al. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 1999;11:81-8.
9. Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, et al. Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001;107:535-41.
10. Sekiya T, Miyamasu M, Imanishi M, Yamada H, Nakajima T, Yamaguchi M, et al. Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells. *J Immunol* 2000;165:2205-13.
11. de Lavareille A, Roufosse F, Schandené L, Stordeur P, Cogan E, Goldman M. Clonal Th2 cells associated with chronic idiopathic hypereosinophilia: TARC-induced CCR4 down-regulation in vivo. *Eur J Immunol* 2001;31:1037-46.

Bound volumes available to subscribers

Bound volumes of The Journal of Allergy and Clinical Immunology are available to subscribers (only) for the 2002 issues from the Publisher, at a cost of \$107.00 for domestic, and \$128.00 for international subscribers for Vol. 109 (January-June) and Vol. 110 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887; phone (800) 654-2452 or (407) 345-4000.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.