

Review article

Recent advances in pathogenesis and management of hypereosinophilic syndromes

Idiopathic hypereosinophilic syndrome is a largely heterogeneous disorder defined until now as persistent marked hypereosinophilia of unknown origin generally complicated by end-organ damage. Recent studies clearly indicate that many patients fulfilling the diagnostic criteria of this syndrome can now be classified as presenting one of two major disease variants: the myeloproliferative or the lymphocytic variant. Research in cellular and molecular biology has provided firm evidence for the existence of discrete hematological disorders underlying these variants, questioning the pertinence of continued reference to 'idiopathic' hypereosinophilic syndrome in such patients. Furthermore, identification of these variants has a number of prognostic and therapeutic implications that must be taken into consideration for adequate management of these patients.

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Key words: imatinib; interleukin-5; lymphoma;
myeloproliferative.

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Accepted for publication 12 November 2003

Hypereosinophilia is a common biological finding in clinical practice, and can be ascribed to an underlying disease in the majority of cases. Although parasitic diseases involving tissue-invasive helminths and atopy or allergic drug reactions account for most cases of hypereosinophilia, a number of less frequently encountered diseases may be implicated, including hematological malignancies, solid tumors, connective tissue disorders, vasculitis, infectious disease, and cutaneous disorders. Thus, when dealing with a patient presenting persistent hypereosinophilia, the clinician's major task is to identify underlying disease, and therapeutic options are quite straightforward. Occasionally, however, thorough diagnostic evaluation fails to reveal a disease known to be associated with hypereosinophilia. Depending on clinical presentation, diagnosis of relatively well-defined organ-specific idiopathic hypereosinophilic disease (i.e. eosinophilic gastro-enteritis, chronic eosinophilic pneumonia, Well's syndrome, Gleich's syndrome, etc.) or of idiopathic hypereosinophilic syndrome (IHES) is considered. It is believed that clinical manifestations and complications encountered in such patients are a direct result of eosinophil accumulation, as this leukocyte subset is known for its highly toxic content. In these disorders, the clinician's major task, therefore, consists in controlling eosinophil levels, which may represent a true challenge. In this paper, we will revisit the management of IHES in light of current insight regarding pathogenic heterogeneity of this disease.

Historical overview of the clinical definition of HES

Depending on the sites and severity of organ damage associated with persistent idiopathic hypereosinophilia, various disease entities have been defined in past literature, including Löffler's syndrome (isolated lung involvement), Löffler's endocarditis parietalis fibroplastica with blood eosinophilia (cardiac involvement), disseminated eosinophilic collagen disease, and eosinophilic leukemia. The frequency of overlapping clinical presentations and heterogeneity within each entity made specific diagnosis in a given patient rather arbitrary. In an attempt to address chronically hypereosinophilic patients with organ damage as a whole, Hardy and Anderson proposed the concept of 'hypereosinophilic syndromes' (HES) in 1968, stressing the frequency of heart involvement (1).

In 1975, Chusid et al. established the empirical diagnostic criteria of 'idiopathic HES' (IHES) that are still in use today: (i) blood eosinophilia exceeding $1500 \mu\text{l}^{-1}$ for more than six consecutive months; (ii) absence of an underlying cause of hypereosinophilia despite extensive diagnostic evaluation; and (iii) presence of organ damage or dysfunction related to hypereosinophilia (2). Published patient series based on Chusid's diagnostic criteria have consistently shown that major tissue targets in HES include the skin, heart, and nervous system, with more than 50% of patients presenting clinical complications in each of these sites (3, 4). Cutaneous manifestations generally consist either in angioedematous and urticarial lesions, or erythematous, pruritic papules, and nodules.

Mucosal ulcerations have also been reported in a number of cases. Cardiac involvement generally evolves in three stages. The early necrotic stage, rarely symptomatic, is followed by a thrombotic stage in which intracavitary thrombi develop along the damaged endocardium. In the final fibrotic stage, endomyocardial fibrosis and damage of atrioventricular valves result in congestive heart failure. Neurological complications involve both central and peripheral nervous systems. Eosinophilic infiltration of other target organs including lungs, liver, spleen, digestive system, articulations, and kidneys may result in a variety of clinical manifestations. Peripheral thromboembolic phenomena independent from those related to endomyocardial lesions may occur. Chusid's initial paper, and all series that have followed, clearly illustrate the great clinical heterogeneity and highly variable prognosis of IHES, ranging from paucisymptomatic disease requiring no treatment and associated with prolonged survival, to rapidly fatal disease course due to the sudden development of congestive heart failure or to the occurrence of acute leukemic disease.

Increased awareness of the critical role played by eosinophils in the development of tissue damage has fuelled recommendations that idiopathic hypereosinophilia be treated rapidly and aggressively in the presence of life-threatening organ involvement. As a result of prompt medical intervention, some patients experience normalization of eosinophil levels before the defined 6-month interval is completed, and thus do not fulfill the duration criterion of Chusid's definition although they clearly present IHES. This has led several authors to suggest that criteria be revised to include such patients.

Finally, in the past few years, much progress has been made toward understanding pathogenesis of this disorder, as will be developed in the following sections. With the discovery of different well-characterized underlying molecular defects ultimately leading to eosinophil expansion in patient sub-populations, the term 'idiopathic' is outdated in many cases.

Pathobiology of hypereosinophilia

Accumulation of eosinophils in peripheral blood and tissues can either be the result of an acquired abnormality involving the myeloid lineage (primary eosinophilia), or be due to the production of eosinophilopoietic cytokines by nonmyeloid cells (secondary eosinophilia) (5). In primary eosinophilia, clonal expansion may occur late in the process of eosinophilic differentiation, in which case the rare diagnosis of 'eosinophilic leukemia' is currently thought to be appropriate. If the abnormality occurs at an earlier stage of differentiation, eosinophils appear to be part of a malignant clone also including other members of the myeloid (and occasionally lymphoid) lineage in the setting of a given myeloprolif-

erative (MP) disorder (i.e. chronic myelo-monocytic leukemia, etc.). In secondary hypereosinophilia, polyclonal eosinophil accumulation is a reactive cytokine-driven process.

Eosinophils are derived from myeloid progenitors (GEMM-CFU) in bone marrow, through the action of three hematopoietic cytokines, GM-CSF, interleukin (IL)-3, and IL-5, among which only the latter is specific for eosinophil differentiation (6). Mature eosinophils are released into the blood stream and rapidly migrate to peripheral tissues, namely gut and bronchial mucosae and skin, where survival is short unless apoptosis is prevented by factors such as IL-3, IL-5, and/or GM-CSF. Thus, overproduction of one or more of these cytokines is sufficient to induce blood and tissue eosinophilia through both stimulation of bone marrow generation, and inhibition of peripheral destruction.

In human pathology, dysregulated production of one or more of these cytokines by abnormal or physiological cell populations accounts for hypereosinophilia in various disorders. Hence, malignant cells producing GM-CSF, IL-3, and/or IL-5 are responsible for hypereosinophilia observed in some patients with non-Hodgkin's lymphoma, Sezary syndrome, and solid tumors such as lung cancer (3, 7, 8). In allergic disorders and parasitosis, the role of IL-5 in the induction of hypereosinophilia is now well established (9). Although several cellular sources of IL-5 have been identified (including mastocytes, basophils, and eosinophils), helper (CD4⁺) T-cells displaying a 'type 2' cytokine profile appear to be primarily involved in these disorders (10). These so-called Th2 cells produce a variety of cytokines in addition to IL-5, including IL-4 and IL-13, which are responsible for induction of IgE synthesis. In clinical practice, Th2-mediated immune responses are thus characterized by the association of hypereosinophilia and high serum IgE levels. Polyclonal hypergammaglobulinemia is also a frequent finding in this setting, due to polyclonal B-cell activation favored by the Th2 climate.

Whatever its cause, eosinophil accumulation is deleterious and may in itself have pathological consequences as a result of local release of toxic substances, including cationic proteins, enzymes, reactive oxygen species, pro-inflammatory cytokines and arachidonic acid-derived factors (6).

Pathogenically distinct variants of HES

The striking clinical heterogeneity among patients with IHES and the occasional development of malignancy involving either the myeloid or the lymphoid lineage strongly suggest pathogenic diversity. Recent observations indicate that distinct primitive hematological disorders involving either myeloid or lymphoid cells account for hypereosinophilia in patients fulfilling the initial diagnostic criteria of IHES.

The myeloproliferative variant

Early investigators singled out a subgroup of patients with clinical and biological features reminiscent of chronic myelogenous leukemia (CML) and other MP syndromes (including increased serum vitamin B12, altered leukocyte alkaline phosphatase score, chromosomal abnormalities, anemia and/or thrombocytopenia, hepatomegaly, splenomegaly, circulating leukocyte precursors) as presenting a more aggressive disease variant, qualified as the 'myeloproliferative variant' of IHES (m-HES) (11, 12). Prognosis has been considered poor in this clinically defined variant, given the frequent occurrence of severe cardiac complications, resistance to glucocorticoid (GC) therapy, and the increased risk of developing myeloid malignancy. Indeed, a proportion of patients with m-HES were known to develop blast crisis clinically reflected either by acute (eosinophilic or myeloid) leukemia or by granulocytic sarcoma.

Recent molecular evidence for a primitive myeloid disorder. Existence of a true MP disorder in a subgroup of IHES patients remained purely speculative at the time, as initial investigators were unable to detect a clonal myeloid disorder responsible for eosinophilic expansion. Demonstration of eosinophil clonality has proven difficult, with only a few reports showing chromosomal abnormalities adjacent to eosinophil granules (13) or skewed methylation patterns of X-linked genes such as phosphoglycerate kinase (PGK) (14) and human androgen receptor (HUMARA) (15) in purified granulocytes and eosinophils, respectively. In practice, these methods are rarely applicable in IHES patients, as karyotype abnormalities are rare in this disorder, and X-linked polymorphisms can be investigated only in female patients, in the setting of a disease that predominantly affects males.

More recently, an outstanding study has provided firm cytogenetic evidence for the existence of a clonal MP disorder in a subgroup of HES patients, and has paved the way for a whole new area of research. In this study (16), the observation of the karyotypic abnormality t(1;4)(q44;q12) in a patient who responded to imatinib mesylate, a tyrosine kinase inhibitor used to treat patients with CML (17), instigated detailed analysis of chromosome 4q12. An interstitial deletion on 4q12 resulting in fusion of FIP1L1 and PDGFR α genes was detected, and was then shown to be present in a further eight of 15 IHES patients with apparently normal chromosome 4 according to routine karyotypes. The fusion gene is in-frame and encodes a FIP1L1-PDGFR α (F/P) protein displaying constitutive tyrosine kinase activity. Similar findings have now been confirmed by other groups both on patient samples (18) and in the EOL-1 cell line (19) derived from blood of a patient who developed acute eosinophilic leukemia following diagnosis of IHES. It is likely that the mutation occurs early in the process of myeloid differentiation, as indicated by its presence in

other cells derived from myeloid progenitors (20). The central role of this fusion gene in disease pathogenesis is supported by its disappearance in most patients successfully treated with imatinib (20, 21). Furthermore, its transforming potential is indicated by its ability to render the murine hematopoietic cell line Ba/F3 IL-3-independent *in vitro* (16, 19) and its presence in the EOL-1 cell line (19).

It is likely that several cytogenetically distinct primitive myeloid disorders are involved in the pathogenesis of m-HES. Indeed, among IHES patients responding to imatinib mesylate, only about two-thirds present the F/P fusion gene (16), suggesting involvement of other as of yet unidentified tyrosine kinase gene(s) in the induction of hypereosinophilia in the remaining third. Future directions for research include identification of additional intrachromosomal rearrangements leading to tyrosine kinase-dependent clonal eosinophil expansion. In other patients, eosinophil expansion may be dependent on other as of yet unknown mechanisms that are not inhibited by imatinib mesylate. Future investigations could focus on genes for membrane growth receptors or for signaling pathways between the membrane and the nucleus, as mutations at this level are typically implicated in chronic MP disorders as opposed to acute presentations.

Tentative definition of m-HES and overlap with chronic eosinophilic leukemia. Despite increasing interest in defining pathogenic variants of IHES, no unifying definition for m-HES has yet been proposed. At this point in time, two major groups of patients could be distinguished depending on whether or not a clonal myeloid disorder (i.e. eosinophil clonality) has clearly been demonstrated. The first group would include patients with the F/P fusion (and any other clonal cytogenetic abnormality yet to be discovered) and those in whom eosinophil clonality has been demonstrated using other methods such as analysis of X-linked polymorphisms. The second group ('presumed m-HES') would include patients with a defined number of features strongly suggestive of an MP disorder. In a recent paper, Klion et al. considered that diagnosis of m-HES was appropriate when four of eight laboratory criteria were fulfilled (i.e. presence of dysplastic eosinophils, increased serum B12, increased serum tryptase, anemia/thrombocytopenia, increased bone marrow cellularity with left shift, myelofibrosis, and dysplastic mast cells or megakaryocytes in bone marrow) (21). Additionally, it is likely that patients responsive to imatinib in whom the F/P mutation is not disclosed present m-HES.

Whether patients with m-HES, according to modern methods in molecular biology, should still be considered as presenting IHES at all is debatable, and the more appropriate diagnosis of chronic eosinophilic leukemia (CEL) has been recommended for those in whom eosinophil clonality, clonal cytogenetic abnormalities

within cells of the eosinophil lineage, and/or increased blasts can be demonstrated (5, 22). Until recently, a limited number of well-defined chromosomal abnormalities encountered in specific subtypes of chronic MP disease associated with hypereosinophilia were considered as markers of CEL. These abnormalities consist in translocations resulting in the creation of fusion genes with oncogenic potential, similar to the BCR-ABL fusion gene resulting from t(9;22) in Philadelphia (Ph)-chromosome-positive CML. Examples include t(5;12)(q33;p13) and t(8;13)(p11;p12) with their associated ETV6-PDGFR β and ZNF198-FGFR1 fusion genes, respectively, the first of which is thought to mediate leukemogenesis through constitutive activation of the PDGFR β tyrosine kinase (5).

However, abnormal karyotypes are rare occurrences in the workup of patients with IHES. Description of the cryptic F/P fusion on chromosome 4q12 in a subgroup of patients has now extended possibilities of identifying those with myeloid clonality, and has led several investigators to consider CEL as a more accurate diagnosis for such patients despite the absence of other indicators of leukemic disease (i.e. blastosis). Indeed, their illness is closer to CML in that clonal expansion is the result of constitutive tyrosine kinase activation, making them likely to respond to similar therapeutic strategies, and both entities present a risk of acute transformation. In contrast, demonstration of eosinophil clonality solely on the basis of nonrandom X chromosome inactivation appears to pick up patients with a very different profile in some cases.

Diagnosis of CEL in patients initially fulfilling diagnostic criteria of IHES should thus be reserved for those in whom myeloid (eosinophil) clonality associated with specific clonal cytogenetic rearrangements resulting in oncogene expression can be demonstrated using methods such as interphase fluorescence *in situ* hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR). However, in the scope of this review article, we will continue to use the term m-HES for such patients.

Clinical profile of patients with m-HES. Based on the probable diversity of primitive disorders underlying m-HES, patients regrouped under this term present a certain degree of heterogeneity with regard to clinical presentation, prognosis, and response to therapy. Indeed, two patients in whom eosinophil clonality was demonstrated on the basis of X-linked polymorphisms had a distinct clinical profile (neither presented features of MP disease, whereas they both had increased serum IgE levels and responded strikingly well to GC therapy) (14, 15) compared to patients in whom the F/P fusion was detected by FISH and/or RT-PCR (see below). This may be related, in part, to gender-specific subtypes of m-HES, as the former are necessarily female patients, and the latter are largely males.

Male predominance of IHES associated with features of MP disease was already reported by initial investigators (2, 4). Occurrence of the F/P mutation in a large majority of male patients probably accounts for this clinical observation (16, 18). Interestingly, other chronic MP disorders associated with hypereosinophilia appear to present an extreme male bias, such as those characterized by translocations involving 5q31-33 (23). Whether this reflects hormonal effects on the creation of specific genetic breakpoints or on expression of certain fusion genes, or requirement of a second X-linked mutation for clinical expression, remains unknown.

Other clinical and biological features frequently encountered in m-HES of interest to clinicians include anemia and/or thrombocytopenia, increased serum B12 levels, mucosal ulcerations, endomyocardial fibrosis, and splenomegaly. Obviously, some of these elements are defining features for 'presumed' m-HES in patients in whom clonal cytogenetic abnormalities have not been disclosed (see tentative definition). As for patients with the F/P fusion, their presence has been demonstrated in a large proportion of cases (18, 24). Thus, recent observations on patients with molecular evidence for eosinophil clonality have confirmed the long-held notion that m-HES is associated with more severe complications of hypereosinophilia, and that the heart is a major target of eosinophils in this variant.

The mechanisms responsible for increased frequency of fibrotic complications in m-HES are largely unknown, although recent observations on a series of patients classified as m-HES, on the basis of increased serum tryptase levels and presence of the F/P fusion, have indicated that mast cells may be implicated in this process (18). Bone marrow biopsies have shown increased numbers of atypical spindle-shaped mast cells and associated myelofibrosis in these patients, which may account for presence of myeloid precursors in peripheral blood and decreased differentiation of red blood cells and platelets. It is likely that the expanded atypical mast cell population is part of the F/P fusion-positive clone. Indeed, although the F/P fusion has not been formally demonstrated within mast cells in patients with HES, the spindle-shaped mast cells disappear in those that respond to imatinib (21). Furthermore, its presence has been detected in three patients with systemic mastocytosis associated with hypereosinophilia (SMCD-eos), suggesting that mast cell expansion may indeed accompany eosinophil expansion in HES patients with the F/P fusion (20). Although mechanisms of mast cell involvement in development of fibrosis remain largely unknown, it has been suggested that mast cell-derived tryptase could serve as a mediator between clonal eosinophils and fibroblasts (18, 21).

Although frequent occurrence of 'myeloproliferative features' in patients with the F/P mutation have been confirmed in most studies, it must be kept in mind that clinical presentation may occasionally be strikingly different, such as that observed in one patient with high

IgE levels, cutaneous manifestations, bronchial hyperre-activity, absence of cardiac involvement, and good response to very low doses of GCs (19).

The lymphocytic variant

Early studies showing that T-cell clones derived from peripheral blood of patients with HES displayed eosinophilopoietic activity in the presence of stem cells from healthy subjects have led authors to suggest that T-cells could be involved in disease pathogenesis through the release of soluble factor(s) (25, 26). Emergence of the Th1/Th2 paradigm has rekindled interest in the pathogenic role of T-cells in HES, as the association of hypereosinophilia with increased IgE levels in some patients suggests the possibility of a Th2-mediated disorder.

Evidence for a primitive lymphoid disorder. In 1994, investigation of circulating T-cells isolated from a patient with HES and high serum IgE and IgM levels revealed the existence of an underlying T-cell disorder characterized by clonal expansion of a T-cell population able to produce IL-5 and IL-4, and bearing a unique CD3⁺CD4⁺ (CD2⁺TCR α/β ⁺) surface phenotype (27). Since then, IL-5-producing T-cell subsets have been described in blood of about 35 patients with HES (28–36) (Table 1). The allegedly pathogenic T-cells display an aberrant surface phenotype in all reported cases, and CD3⁺CD4⁺ cells represent the most frequently encountered subset in this setting. A CD3⁺CD4⁺CD8⁺ phenotype, previously reported in patients with Fas mutations in the human autoimmune lymphoproliferative syndrome, has been described in four patients. Whatever their cell surface phenotype, the aberrant lymphocyte subsets generally express surface antigens characteristic of activated (HLA-DR⁺ and/or CD25⁺) memory (CD45RO⁺)

T-cells (28, 29). Other phenotypic features include high-level expression of CD5 by CD3⁺CD4⁺ cells, and frequent loss of surface CD7 and/or CD27 expression.

Clonality of the phenotypically aberrant T-cells has been demonstrated in many cases, and chromosomal abnormalities, including 16q breakage (31), partial 6q or 10p deletions (29), and trisomy 7 (35) have been reported (Table 1). Extensive analysis of the cytokine profile of aberrant T-cells has shown their pathogenic role in induction of hypereosinophilia given their ability to produce IL-5. Furthermore, CD3⁺CD4⁺ cells have been shown to produce the Th2 cytokines IL-4 and IL-13, as well as IL-2. In a recent study, these cells were also shown to produce tumor necrosis factor (TNF)- α and GM-CSF (35). As for interferon (IFN)- γ , aberrant T-cell clones vary in their ability to produce this Th1 cytokine (Table 1).

On the basis of currently available data, the 'lymphocytic variant' of HES (l-HES) can be defined as a primitive lymphoid disorder characterized by nonmalignant expansion of a T-cell population able to produce eosinophilopoietic cytokine(s) (generally IL-5).

Clinical profile of patients with l-HES. In contrast to m-HES, l-HES appears to affect females at least as much as males. Although there exists considerable clinical heterogeneity among patients fulfilling the diagnostic criteria of IHES, those in whom aberrant IL-5-producing T-cells have been detected exhibit a strikingly homogenous clinical and biological profile (Table 2). Cutaneous manifestations, including pruritus, eczema, erythroderma, urticaria, and angioedema, are observed in virtually all patients reported in the literature (28, 29). A previous history of typical atopic disease is frequently encountered. In contrast, very few patients with l-HES develop endomyocardial fibrosis despite high eosinophil levels. Complications of hypereosinophilia in this subgroup of

Table 1. Characteristics of phenotypically aberrant T-cell subsets in l-HES patients

	Number of patients	Aberrant phenotype	T-cell clonality		Cytokine profile	Cytogenetic abnormality
O'Shea, 1987	2	CD3 ⁺ CD4 ⁺	nd	nd		nd
Bagot, 1990	1	CD3 ⁺ CD4 ⁺	+	nd		nd
Moraillon, 1991	1	CD3 ⁺ CD4 ⁺	nd	nd		nd
Kitano, 1996	1	CD3 ⁺ CD4 ⁺ CD8 ⁺	+	IL-5, GM-CSF		Chrom 16
Simon, 1999*	16	Variable†	8/16	IL-5 (13/16); IL-4 (4/16)		nd
Zenone, 1999	1	CD3 ⁺ CD4 ⁺	+	nd		NL
Roufosse, 2000‡	4	CD3 ⁺ CD4 ⁺	4/4	IL-2, IL-4, IL-5 (4/4) IFN- γ (1/4)		Chrom 1,6,10 (3/4)
Bank, 2001§	3	CD3 ⁺ CD4 ⁺	2/3	IL-4, IL-5, GM-CSF		nd
Sugimoto, 2002	1	CD3 ⁺ CD4 ⁺	+	IL-4, IFN- γ		t(2 ;14), t(12 ;14)
Roumier, 2003	1	CD3 ⁺ CD4 ⁺	+	IL-2, IL-4, IL-5, IL-13, TNF- α , GM-CSF, IFN- γ		Chrom 7
Roufosse, unpublished	1	CD3 ⁺ CD4 ⁺	+	IL-2, IL-4, IL-5, IL-13, TNF- α , GM-CSF, IFN- γ		Chrom 17

* Patients in refs 29 and 38 included in this series; CD3⁺CD4⁺CD8⁺ cells in one patient (ref. 38) also produced the eosinophilopoietic cytokine IL-3.

† Including CD3⁺CD4⁺CD8⁺ cells ($n = 3$), CD3⁺(or low)CD4⁺ cells ($n = 2$), and decreased or increased expression of CD2, CD5, CD6 or CD7 on CD4⁺T-cells.

‡ Patient in ref. 26 included in this series.

§ One patient in this series was previously reported in ref. 28 (Roufosse, 2000, in the table), and therefore not included in this table.

NL, normal; nd: not done.

Table 2. Clinical profile of I-HES patients with aberrant T-cells populations

	Sex	Age* range (mean)	Routine biology			Clinical complications		
			Eosinophil level† (per μ l)	Serum IgE	Serum Ig(GAM)	Skin	Other‡	Lymphoma
O'Shea, 1987	1F; 1M	37; 27	13 000; 5000	NL; $\uparrow$	NL; $\uparrow$	2/2	L, V	2/2
Bagot, 1990	1M	13	4000	$\uparrow\uparrow$	nd	+	—	1
Moraillon, 1991	1M	35	25 000	$\uparrow$	$\uparrow$	+	—	1
Kitano, 1996	1M	53	6270	nd	nd	—	H	—
Simon, 1999§	11F; 5M	5–88 (59)	869–5805	$\uparrow$ 8/16	nd	14/16	L, GI	3/16
Zenone, 1999	1F	38	2200	NL	$\downarrow$	+ (Gleich S.)	—	—
Roufosse, 2000¶	3F; 1M	16–34 (32)	2970–9100	$\uparrow$ 4/4	$\uparrow$ 4/4	4/4	L, V, Rh	2/4
Bank, 2001**	2F; 1M	38–70 (56)	4800–7000	$\uparrow$ 2/3	nd	3/3	L, Rh, TE	1/3
Sugimoto, 2002	1F	55	13 700	$\uparrow$	NL	+	L	—
Roumier, 2003	1M	20	9500	$\uparrow$	$\uparrow$	+	—	—
Roufosse, unpublished	1M	34	5350	NL	$\uparrow$	+ (Gleich S.)	Rh	—

NL, normal; nd, not done.

* Age at presentation and discovery of hypereosinophilia.

† Highest eosinophil level reported.

‡ L, lung; V, vasculitis; H, heart; GI, gastro-intestinal; Rh, rheumatological (arthritis, tenosynovitis); TE, thromboembolic.

§ Patients in refs 29 and 38 included in this series.

¶ Patient in ref. 26 included in this series.

** One patient in this series was previously reported in ref. 28 (Roufosse 2000), and therefore not included in this table.

patients more commonly occur in the lungs and the digestive system. Although skin is involved in many IHES patients regardless of disease variant, the relative paucity of associated organ involvement in patients with an underlying T-cell disorder makes cutaneous manifestations a cardinal clinical feature in such patients.

Skin biopsies in patients with aberrant T-cell subsets and cutaneous manifestations show prominent eosinophil accumulation. However, no study to date has characterized adjacent infiltrating T-cells, leaving the question open whether the IL-5-producing T-cells display epidermotropic behavior, thereby inducing preferential eosinophil migration to skin. Although CD3⁺CD4⁺ cells have been shown not to express cutaneous lymphocyte antigen (unpublished observations), they consistently lack surface CD7 antigen, a characteristic of skin-homing T-cells (37). Interestingly, the principal target organs in this HES variant (i.e. skin, lungs, digestive tract) are those in direct contact with environmental stimuli, and are typically involved in allergic disorders.

Biologically, in accordance with the type 2 cytokine profile of the aberrant T-cells, serum IgE levels are often increased and polyclonal hypergammaglobulinemia, principally due to increased IgM and/or IgG levels, is observed in some cases (28, 29). Finally, among hypereosinophilic patients with an IL-5-producing CD3⁺CD4⁺ T-cell clone, some (29, 33) present a clinical profile indistinguishable from that encountered in Gleich's syndrome (38), or episodic angioedema with eosinophilia, a disease characterized by spontaneously remitting episodes of angioedema, high serum IgM levels, and hypereosinophilia.

Pathogenesis of I-HES. The pathogenesis of T-cell clonality in the setting of I-HES remains unknown. Simon

et al. have shown that the aberrant T-cell population lacked CD95/Fas-R expression in 8/16 patients of their series (28), and demonstrated that deficient Fas-mediated apoptosis was involved in expansion of a CD3⁺CD4⁺CD8⁺IL-5-producing lymphocyte subset in one such patient (39). However, CD3⁺CD4⁺ cells from our patients expressed Fas-R and were highly sensitive to apoptosis following engagement of this receptor *in vitro*, as assessed by *in vitro* exposure to soluble Fas-ligand (40), suggesting that primary events leading to clonal T-cell expansion can differ among hypereosinophilic patients with an underlying T-cell disorder.

The highly similar functional characteristics of aberrant CD3⁺CD4⁺ T-cells reported in the literature and the homogenous clinical profile associated with their presence indicate the possibility of a common pathogenic agent. Absent or low expression of the TCR/CD3 complex by CD4⁺ T-cells has been observed in several pathological settings including (retro-)viral infections (41–43) and chronic antigenic stimulation (44). In our experience, all patients with CD3⁺CD4⁺ T-cells tested negative for HIV and HTLV₁ serology. HTLV₁ and HTLV₂ proviral sequences were not detected in CD3⁺CD4⁺ T-cells from three patients in our series, and antibodies against Tax were not detected in their serum (unpublished observations). Furthermore, the CD3-negative phenotype of clonal T-cells from our patients was stable even after prolonged culture in the presence of rIL-2 *in vitro* (unpublished observations) indicating that the loss of TCR/CD3 expression did not depend on continuous exposure to a putative exogenous antigen *in vivo*. Detection of intracellular CD3- ϵ and TCR- α/β chains in clonal cells suggests defective membrane translocation of the TCR/CD3 complex, a process known to be dependent on

the conserved expression of the different TCR- and CD3-chains (45).

Prognosis of HES variants

Prognosis of IHES as a whole is dependent on the severity of end-organ damage, especially heart involvement, and on the development of malignancy. With increased awareness of the necessity of lowering eosinophil levels to prevent target organ damage, and with better surgical management of cardiac complications, survival rates have improved dramatically, passing from 3-year survival of 12% in Chusid's initial 1975 retrospective study (average survival of 9 months) to 10-year survival of 70% in Fauci's prospective 1982 study. However, occurrence of malignant hematological disorders has remained a subject of concern.

The myeloproliferative variant

Target organ damage, especially heart failure due to endomyocardial fibrosis, was a major determinant of poor overall prognosis of IHES reported in initial studies, and was clearly associated with features of MP disease (4). Recent data have shown that cardiac complications are preferentially observed in patients with elevated serum tryptase levels (18), which appear to be indicative of an underlying F/P fusion, lending support to the long-held notion that the heart is a major target of eosinophils in m-HES. Importantly, endomyocardial fibrosis due to persistent hypereosinophilia is irreversible, as shown by persistence of clinical manifestations and echographic abnormalities in patients whose eosinophil levels and other complications are well controlled by treatment with imatinib (21, 24). It is, therefore, critical that eosinophil levels be controlled before development of cardiac complications.

Early identification of patients with an increased likelihood of developing irreversible fibrotic complications of hypereosinophilia would provide clinicians with a means of selecting those warranting aggressive management of eosinophil levels. Measurement of serum tryptase levels may be helpful in this regard, as increased levels were shown to be associated with the development of endomyocardial fibrosis and occurrence of disease-related death (18). The value of serum tryptase as a prognostic marker remains to be evaluated on a larger scale.

As for the development of a malignant disorder, follow-up of patients with m-HES has shown that some will experience acute blastic transformation (3, 5), manifesting either as acute myeloid leukemia (AML), or as granulocytic sarcoma (46). Given the recent description of the F/P mutation, there are little data available concerning malignant progression in this group of patients. However, at least two patients with this fusion gene developed leukemia (19, 24). Demonstration of the

transforming potential of the F/P fusion *in vitro* provides a basis for these observations, although the succession of events at the molecular level remains entirely to be elucidated.

As a whole, presence of the F/P mutation is indicative of a subgroup of m-HES patients with poor prognosis and a high prevalence of disease-related death due both to development of cardiac complications and to increased risk of developing AML. It can be hoped that timely administration of imatinib to these patients will modify the disease course and delay or prevent malignant transformation.

The lymphocytic variant

Although reports on the clinical profile of patients with an IL-5-producing T-cell population have confirmed the rarity of end-organ damage (in particular heart involvement) and good short-term prognosis compared to m-HES, they have shown that long-term prognosis may be less favorable than once thought due to occurrence of T-cell malignancy.

Indeed, identification of phenotypically aberrant T-cells in peripheral blood of patients with HES may be followed by protracted development of peripheral T-cell lymphoma in some cases (28, 29, 36, 47). Importantly, the lymphomatous cells have been shown to conserve the abnormal phenotype in a few patients, indicating that the initially observed aberrant T-cells may be premalignant precursors. Additional cases wherein aberrant circulating T-cell clones are discovered simultaneously with diagnosis of peripheral T-cell lymphoma further support these observations (48, 49). Repeated studies of CD3⁺CD4⁺ cells during a period of several years in one patient who eventually developed lymphoma have indicated that the aberrant cells do indeed undergo a process of progressive transformation, as assessed by a series of morphological, phenotypic, and cytogenetic changes (unpublished data). Clinically, these events occurred simultaneously with increasing CD3⁺CD4⁺ lymphocytosis, and the appearance of enlarged lymph nodes harboring CD3⁺CD4⁺ lymphomatous cells with important metabolic activity as assessed by fluoro-deoxyglucose positron-emission tomography (FDG PET).

Thus, l-HES appears to be a benign clonal disorder with indolent behavior, progressing to full-blown malignancy in only a proportion of cases. The nature of phenotypic surface abnormalities on T-cells encountered in a given patient may constitute a risk factor for the development of malignancy. Indeed, it can be inferred from existing literature that the loss of surface TCR/CD3 expression by CD4⁺ cells is associated with malignant transformation, as CD3⁺CD4⁺ cells have been detected in patients with ataxia-telangiectasia (50) and angioimmunoblastic lymphadenopathy (51), both associated with the development of T-cell lymphoma. In the latter as in l-HES, the CD3⁺CD4⁺ phenotype may remain a

characteristic of the malignant cells in patients who develop lymphoma. Occurrence of T-cell lymphoma in one patient with $CD3^+CD4^-CD8^-$ cells (28) indicates that other phenotypically aberrant cell populations may also have premalignant potential.

Several studies indicate that abnormal expression and/or function of specific surface antigens on these aberrant T-cell subsets may expose them to uncontrolled clonal expansion as a result of impaired negative regulation. In the case of $CD3^-CD4^+$ cells, it has been shown that antigen-presenting cells are able to induce their proliferation *in vitro* despite their inability to recognize allogeneic major histocompatibility complex (MHC) molecules, through engagement of CD2 and CD28 surface molecules and initiation of an autocrine and probably paracrine IL-2/IL-2R- α loop (52). Preliminary data have indicated that TCR-independent stimulation of the $CD3^-CD4^+$ cells may impair retention of cytotoxic T-lymphocyte antigen (CTLA)-4 on the cell surface, leading to deficient

negative regulation of IL-2-dependent clonal proliferation of these cells (unpublished observations). Over time, this may favor accumulation of chromosomal abnormalities and eventually development of T-cell lymphoma (Fig. 1). As for $CD3^+CD4^-CD8^-$ cells, deficient Fas-mediated apoptosis may account for uncontrolled clonal proliferation (39).

The nature of the cytogenetic changes observed in aberrant T-cells from patients with HES may constitute an additional prognostic marker. Indeed, partial 6q deletions have been observed in $CD3^-CD4^+$ cells from two patients with l-HES, preceding the development of T-cell lymphoma in one case (29). In patients with malignant T-cell disorders, such as peripheral T-cell lymphoma and adult T-cell leukemia/lymphoma, 6q deletions are thought to be associated with poor prognosis due to the loss of tumor suppressor genes (53, 54). Our observation that $CD3^-CD4^+$ cells, which displayed stable chromosomal changes (partial 6q and 10p

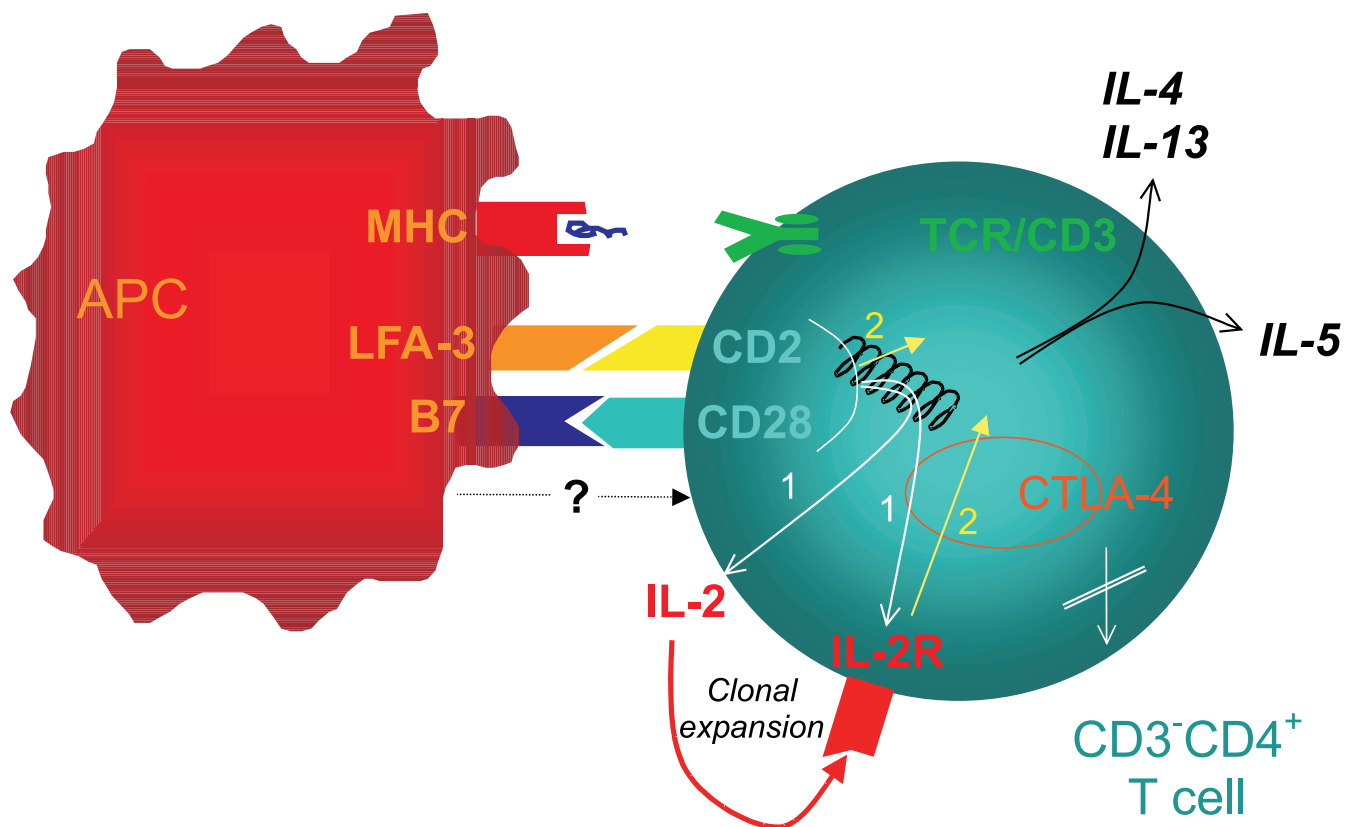


Figure 1. $CD3^-CD4^+$ T-cells associated with l-HES proliferate and produce type 2 cytokines in response to antigen presenting cells *in vitro*. In a previous publication (51), it was shown that $CD3^-CD4^+$ T cells isolated from patients with l-HES could be activated *in vitro* by LPS-matured allogeneic dendritic cells (DC), despite their inability to recognize foreign MHC molecules. Engagement of CD2 and CD28 molecules on the T-cell surface by corresponding LFA-3 and B7 ligands on DCs results in upregulation of IL-2 secretion and IL-2R α (CD25) expression by $CD3^-CD4^+$ cells. Signaling through CD2, CD28, CD25 and presumably ligation of other unknown soluble molecule(s) produced by DCs result in enhanced survival and proliferation of the $CD3^-CD4^+$ T-cells as well as production of type 2 cytokines. Activation of $CD3^-CD4^+$ T-cells in response to DCs is not followed by upregulation of CTLA-4, a negative regulator of T-cell expansion. This may contribute to uncontrolled expansion of the aberrant T-cell population.

deletions) for several years in one patient with l-HES, had developed a number of secondary mutations when full-blown peripheral lymphoma was diagnosed (unpublished data) are in keeping with Collins' suggestion that accumulation of genomic aberrations may be a reliable sign of malignant progression in clonal lymphocytic disorders (55).

The evidence for increased risk of lymphoid malignancy in patients with the recently defined l-HES challenges the long-held notion that isolated cutaneous manifestations associated with serum hyper-IgE and/or polyclonal hypergammaglobulinemia, in the setting of the IHES, are markers of good prognosis (3, 4). Moreover, identification of clonal CD3⁺ CD4⁺ cells in blood of two patients whose clinical presentations were compatible with Gleich's disease (29, 33) indicates that this reputedly benign idiopathic hyper eosinophilic disorder may also be associated with an increased risk of malignant transformation.

Management of patients with IHES

HES are a rare and complex group of diseases, which represent a precious model for studying the pathogenesis of both MP and lymphoproliferative disorders. Optimal management of patients depends on the ability to classify their disease variant, which requires a certain level of expertise on both technical and clinical levels, as will be discussed in the following section. Moreover, therapeutic perspectives implicate the use of certain molecules or techniques that are not widely distributed among practitioners but rather are assessed in clinical trials. For all these reasons, patients diagnosed as IHES should be referred to experienced teams.

Approaching diagnosis of HES variants

Distinction of an underlying myeloid vs lymphocytic disorder is a critical first step in establishing optimal guidelines for patient follow-up and treatment. We have seen that predominant cutaneous manifestations in the absence of heart involvement, associated with serum hyper-IgE and/or polyclonal hypergammaglobulinemia, should arouse suspicion of l-HES. Likewise, splenomegaly, heart involvement, increased vitamin B12 levels, anemia, and/or thrombocytopenia, and presence of immature myeloid precursors in peripheral blood are indicative of m-HES. However, none of these features are entirely specific of a disease variant, and further testing is warranted in all cases for appropriate categorization of patients. We will review the cellular and molecular investigations that are currently recommended for differential diagnosis of a primitive myeloid vs lymphoid disorder in this setting, and will also discuss cases in which pathogenesis remains unknown following extensive workup.

Table 3. Investigations required for diagnosis of HES variants

<i>Available in most hospital settings</i>	
Complete blood count with leukocyte differential	
Miscroscopic examination of peripheral blood smear	
Serum IgE	
Serum IgG, IgA, IgM	
Serum vitamin B12	
Leukocyte alkaline phosphatase score	
Bone marrow smear and biopsy (tryptase and reticulin stain)	
Lymphocyte phenotyping	
TCR gene rearrangement analysis (Southern blot and PCR)	
Conventional cytogenetic analysis on peripheral blood and bone marrow	
Abdominal ultrasound (measurement of liver and spleen)	
Echocardiogram and cardiac MRI when possible (evaluation of function, and detection of valvular lesions and/or intracavitary thrombus)	
<i>Investigations referred to qualified laboratories</i>	
Serum tryptase	
Serum TARC	
FIP1L1-PDGFR α fusion	
RT-PCR, FISH	
Use of CHIC2 deletion as a surrogate marker remains to be established	
Lymphocyte phenotyping*	
Including CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD25, CD27, CD45RO, TCR α/β , TCR γ/δ , HLA-DR, CD95	
TCR gene rearrangement analysis*	
Eventually on a FACS-sorted phenotypically aberrant population	
Conventional cytogenetic analysis*	
In the presence of rIL-2 in addition to usual mitogens	
Cytokine profile of T cell populations	
IL-4, IL-5, IL-13, IL-3, GM-CSF, IL-2, IFN- α	
Intracellular cytokines at the single cell level by flow cytometry	
Cytokines in culture supernatants of phenotypically suspect T-cell populations	

* When found normal in routine investigations.

Diagnostic evaluation of IHES patients. Initial diagnostic work-up of IHES should include a series of investigations enumerated in Table 3, some of which are routinely available to clinicians, and others that are best referred to specialized laboratories.

Identification of patients with l-HES is based on analysis of circulating T-cells. Currently available literature indicates that lymphocyte phenotyping in the search of a phenotypically aberrant T-cell subset (principally CD3⁺CD4⁺ or CD3⁺CD4⁺CD8⁻), and analysis of TCR gene rearrangement patterns using both Southern Blot and PCR amplification in search of T-cell clonality are generally sufficient to detect patients with an underlying T-cell disorder, and should therefore systematically be performed on peripheral blood and bone marrow samples of all patients with HES. Besides the easily identified grossly aberrant CD3⁺CD4⁺ or CD3⁺CD4⁺CD8⁻ T-cell populations, phenotypic abnormalities of IL-5-producing T-cells are occasionally very discrete, consisting in slight alterations of staining intensity for surface antigens such as CD5, CD6, CD7, and CD27 (28). Special attention should also be paid to markers of functionally activated (HLA-DR, CD25, CD69), memory (CD45RO) T-cells. As for analysis of TCR rearrangement patterns, it has been shown that T-cell clonality is not detected in all patients with a

demonstrated aberrant lymphocyte subset (28). Negative findings may reflect true absence of clonality, or clonality may be undetected due to clonal deletion of TCR chain genes (30), choice of primers in PCRs, or lack of sensitivity when aberrant cells represent a small proportion of total lymphocytes (29). We have shown that repeated testing after the purification of aberrant T-cells revealed clonality that had not been detected with total peripheral blood mononuclear cells (PBMCs) in one such case (29).

Additionally, analysis of T-cell cytokine secretion profiles must be performed for assessment of an underlying Th2-like lymphoid disorder. Recent observations indicate that, even in experienced hands, phenotyping may be normal and search for clonality may be negative in some patients whose PBMC produce increased levels of IL-5 *in vitro* (56). Enhanced production of eosinophilopoietic (IL-3, GM-CSF) and/or type 2 (IL-4, IL-5, IL-13) cytokines by T-cells can be demonstrated either by measuring concentrations in supernatants of cultured PBMCs or purified aberrant lymphocyte subsets by enzyme-linked immuno-sorbant assay, in the absence or presence of T-cell stimulating agents, or by determining the proportion of cytokine-positive cells within a given lymphocyte subset by flow cytometry.

Conventional cytogenetic analysis must be performed systematically in IHES patients, as certain translocations may actually change diagnosis to CEL (i.e. presence of t(5;12)(q33; q13) or t(8;13)), and detection of other chromosomal abnormalities in patients with m-HES or l-HES has prognostic and even therapeutic implications. Karyotyping l-HES patients may require much expertise, as illustrated by the choice of mitogens for aberrant T-cell clones. Indeed, CD3⁻CD4⁺ and CD3⁺CD4⁻CD8⁻ cells respond poorly to stimulation with PHA, favoring outgrowth of normal T-cells during karyotype studies. In one patient with l-HES, chromosome 16 abnormalities were detected in a large proportion of CD3⁺CD4⁻CD8⁻ cells only after addition of IL-2 to PHA cultures (31). Hence, as previously recommended for Sezary cells, growth-promoting cytokines such as IL-2 and/or IL-7 should be added to cultures prior to cytogenetic analysis of aberrant T-cells (54). Importantly, the nature of cytogenetic abnormalities identified in IHES patients may provide insight regarding pathogenesis of disease variants. This has recently been illustrated by the chain of events leading from the observation of a specific translocation t(1;4) in a single hypereosinophilic patient to discovery of the F/P mutation (16).

Given inestimable therapeutic implications, the search for the F/P fusion by RT-PCR and/or FISH is mandatory in all patients with IHES. It has recently been suggested that deletion of the CHIC2 locus, situated between FIP1L1 and PDGFR α on chromosome 4q, could serve as a surrogate marker for the fusion (20). Several teams dealing with IHES patients systematically

search for c-kit and PDGFR β mutations to exclude other MP disorders associated with hypereosinophilia (57).

As for bone marrow sampling, both a smear and biopsy are required (Table 3). First, only biopsies will eventually disclose existence of underlying lymphoma in a patient with chronic hypereosinophilia. In addition, trypan staining may reveal the presence of increased numbers of dysplastic spindle-shaped mast cells and reticulin staining may show degrees of myelofibrosis in m-HES (18).

Classification of patients on the basis of diagnostic evaluation. Ultimately, all patients with IHES present either a primitive myeloid disorder or a primitive lymphoid disorder with reactive eosinophilia. Categorization of a given patient following diagnostic workup can be straightforward in presence of certain results (Table 4). For example, the presence of the F/P mutation is diagnostic of m-HES (or CEL, see WHO criteria (22)), whereas demonstration of an aberrant CD3⁻CD4⁺ T-cell clone able to produce IL-5 signs diagnosis of l-HES. When evaluation fails to reveal clear-cut existence of a primitive myeloid or lymphoid disorder, a third truly 'idiopathic' HES group, which is likely to be short-lived, is defined. It is likely that for most patients in this group, final diagnosis will be possible, either with characteristics of disease progression, or with further identification of molecular abnormalities associated with either myeloid or lymphoid (Th2-like) expansion in the years to come. In the meantime, depending on their clinical and biological characteristics, patients in this third group should be stratified as more or less likely to present myeloid *vs* lymphoid disease (Table 4), and be treated accordingly.

The respective frequencies of HES disease variants have proven difficult to assess given the small size of most published series. In addition, depending on the clinical specialty of each investigator (i.e. specialist in immunology, internal medicine, or hematology), the profile of recruited patients will differ. A first estimation would be approximately one-third of patients present cytogenetically proven m-HES, and one-fourth present clear-cut l-HES.

Identification of diagnostic markers of HES variants. Diagnostic markers for both HES variants that would be reliable and easy to perform on a routine basis would prove highly useful if they could replace the time-consuming and costly work-up that has been outlined above. Furthermore, identification of markers consistently associated with either myeloid or lymphoid expansion could facilitate categorization of patients in the third 'idiopathic' group.

Several markers of MP disease have been investigated in patients with m-HES, including increased serum vitamin B12 levels and serum tryptase levels. Although exceedingly high serum B12 levels appear to be associated with the presence of the F/P fusion (18, 24), a number of unrelated factors may result in increased B12 levels in any

Table 4. Classification of HES patients

Diagnosis of HES on the basis of Chusid's criteria (ref. 2)			
Proven myeloproliferative variant (m-HES)		Unknown pathogenesis (l-HES)	
Proven lymphocytic variant (l-HES)		Presumed lymphocytic variant	
F/P fusion Eosinophil clonality (isolated eosinophils or granulocytes) Nonrandom HUMARA, PGK gene inactivation Abnormal karyotype	Phenotypically aberrant T-cell population	Increased serum B12	Increased serum IgE
	CD3 ⁺ CD4 ⁺	Splenomegaly	Increased serum IgGAM
	CD3 ⁺ CD4 ⁺ CD8 ⁺	Anemia, thrombocytopenia	Predominant cutaneous manifestations
	Other	Increased circulating myeloid precursors	Absence of heart involvement
	Clonal TCR rearrangement pattern	Dysplastic eosinophils	Eventual lung/gastro-intestinal involvement
Increased production of eosinophilopoietic cytokines by T-cells		Increased serum tryptase	Good response to glucocorticoid therapy
Cell culture supernatants		Spindle-shaped mast cells in bone marrow	History of atopy
Flow cytometry		Myelofibrosis	Increased serum TARC
		Significant end-organ damage (heart, nervous system)	
		Imatinib responsive	

individual, making this marker unspecific for m-HES. In contrast, increased serum tryptase appears to be a good marker for m-HES associated with the F/P mutation, as the fusion gene was demonstrated in all patients tested among those with high tryptase levels, but not in patients with normal serum tryptase (18, 21). Absorption of GCs, hydroxyurea (HU), cyclosporine A, and IFN- α appear not to affect tryptase values (18). Whether serum tryptase is increased in patients with myeloid clonality not associated with the F/P mutation, which would make it a valuable marker for diagnosis of m-HES as a whole, remains to be assessed.

As for l-HES, several potential markers of lymphoproliferative and/or Th2 disorders have been assessed in small series. Increased serum IgE is frequently encountered in patients with l-HES, but is neither sensitive nor specific for this variant. Indeed, approximately three-fourths of patients with clonal CD3⁺CD4⁺ cells reported in the literature have high serum IgE levels (36). In the others, normal IgE levels may be due to the inability of aberrant cells to produce IL-4 and IL-13 (30). On the other hand, increased production of IgE is not restricted to patients with l-HES, as observed in one patient with the F/P fusion (19) and in two patients in whom eosinophil clonality was demonstrated by nonrandom X chromosome inactivation (14, 15). Associated eosinophil clonality and increased IgE in such cases may be due to the production of IL-4 and IL-13 by eosinophils themselves. Similarly, several studies indicate that demonstration of increased serum IL-5 levels lacks both sensitivity and specificity for identification of patients with l-HES (29). T-cell-secreted IL-5 may be rapidly consumed by eosinophils and cleared from serum in true l-HES patients, and conversely, it is now clear that activated eosinophils themselves are a potential source of IL-5 (58), which could result in detectable serum IL-5 levels in patients with m-HES. In this line, presumed m-HES patients responding to imatinib had detectable serum IL-5 levels in one study (57), and high serum IL-5 levels have been reported patients with CEL (5) and with chronic MP disease associated with hypereosinophilia and the t(5;12) translocation (23). Serum levels of soluble CD25 (sCD25) appear not to discriminate between HES variants in one preliminary study (29), as both activated T-cells and activated eosinophils may generate this molecule (59).

More recently, the chemokine TARC has gained interest as a potential marker of Th2-mediated disorders (60). One study has shown that serum TARC levels were 10–100-fold higher in 13 patients with l-HES compared to four HES patients with no evidence for an underlying lymphoid disorder, who had levels similar to healthy control subjects (56). GC therapy may interfere with interpretation of results in some cases as we observed rapid normalization of serum TARC levels in one patient with a CD3⁺CD4⁺ T cell clone (unpublished data). These preliminary observations, which remain to be validated in

Table 5. Follow-up of patients with HES

<i>The lymphocytic variant</i>
Quantification of aberrant T-cell population
Analysis of surface markers (CD25, CD69)
Morphology of aberrant T-cells
Conventional cytogenetic analysis (rIL-2 used as a mitogen)
Whole body FDG PET (positron-emission tomography) scan
Biopsy of any suspect lymph node
<i>The myeloproliferative variant</i>
Conventional cytogenetic analysis
Blast counts in blood and bone marrow

a larger patient population, suggest that increased serum TARC levels may represent a highly discriminative diagnostic test for l-HES versus m-HES.

Follow-up of patients with HES variants

With increasing survival of HES patients over time, it is likely that clinicians will witness an increase in the proportion of patients who develop a malignant disorder, namely peripheral T-cell lymphoma in l-HES and AML in m-HES. Although repeated search for malignancy during patient follow-up is recommended, it is currently unclear which investigations will prove useful in this regard. Our limited experience with one patient who developed T-cell lymphoma 6 years after diagnosis of l-HES (CD3⁺CD4⁺ clone) has led us to propose investigations listed in Table 5 as follow-up. Appearance of enlarged lymph nodes should always be followed by biopsy, as lymphomatous cells were detected in lymph nodes in almost all reported cases of lymphoma following diagnosis of HES (36). Besides physical examination of patients, whole body scanning with FDG PET is a sensitive tool for the detection of metabolically active lymph nodes.

Long-term follow-up of patients with the F/P fusion is warranted before similar recommendations can be made in m-HES. Repeated chromosomal analysis in search of increased genetic burden and blast counts in blood and marrow are certainly recommendable.

Therapeutic perspectives in the HES

Therapeutic strategies designed to control eosinophil levels in patients with IHES were defined in 1975 (2) and had changed little until recently. GCs and HU were cornerstones in the management of disease, and IFN- α was introduced in the early 1990s on the basis of several encouraging studies (61). Most molecules used to treat IHES until the beginning of this millennium have been chosen on the basis of the supposedly myelogenous nature of this disorder, and therapeutic strategies have paralleled those proposed for CML. However, with the description of an underlying lymphocytic disorder in a subgroup of patients, and later of a chromosomal rearrangement resulting in the creation of a fusion gene with constitutive

Table 6. Therapeutic strategies in HES variants

<i>Myeloproliferative variant</i>
Imatinib mesylate (Gleevec)
First choice if F/P fusion gene present
Therapeutic trial in presence of features of MP disease
Hydroxyurea
Interferon- α
In presence of signs of malignant transformation
Chemotherapy
Bone marrow or stem cell transplantation
<i>Lymphocytic variant</i>
Glucocorticoids
Interferon- α
Not as monotherapy
Associate with GC
Cyclosporine A
Anti-IL-5 mAb
Remains to be assessed
In presence of signs of malignant transformation
Fludarabine, 2-chlorodeoxyadenosine (2-CdA)
Chemotherapy (CHOP-like regimens)
Bone marrow or stem cell transplantation

tyrosine kinase activity in others, therapeutic perspectives have radically changed in the past 2 years (Table 6).

Therapeutic considerations in m-HES. As expected in MP disorders, patients with m-HES are less likely to respond to GC, but are more likely to respond to HU, IFN- α , and eventually other chemotherapeutic agents such as busulfan, chlorambucil, and vincristine. For patients who develop increased blastosis or other signs of malignant progression, stem cell transplantation has proven effective (3).

A major contribution was made when a few investigators tested effects of imatinib mesylate, an inhibitor of specific protein tyrosine kinases shown to be highly effective in treatment of Ph-chromosome-positive CML (17), on patients with IHES (62). The first published series showed that a low dose of imatinib mesylate (maximum 100 mg/day) had a dramatic effect on eosinophil levels in four of five patients (63). Since then, several independent groups have confirmed that imatinib is a highly effective treatment for a subgroup of HES patients (16, 21, 57, 64), many of whom (approximately two-thirds) present the F/P mutation. *In vitro* data have shown that imatinib not only inhibits phosphorylation of the F/P fusion protein, but also potentially induces apoptosis of cells expressing this protein (19). Given the scientific rationale for treatment of F/P mutation-positive patients with imatinib, and the striking rapidity and potency of its effects in clinical practice, this molecule has rapidly become first-choice treatment for these patients.

Response to therapy in terms of eosinophil levels and clinical manifestations involving skin, digestive system, and lungs is rapid (generally within days) in many cases. In contrast, clinical manifestations related to endomyocardial

fibrosis appear to be irreversible (21, 24). Reversal of bone marrow pathology, as shown by the disappearance of atypical spindle-shaped mast cells and decreased reticulin staining in patients with myelofibrosis prior to therapy, and normalization of serum tryptase levels in parallel, have been demonstrated in a recent study (21). Cytogenetic remission, a major endpoint when dealing with disease mediated by constitutively activated tyrosine kinases, was achieved in several patients with the F/P fusion gene (21, 24).

Interestingly, m-HES patients require lower doses of imatinib (often 100 mg/day, and sometimes even less) than patients with CML to induce and maintain remission. *In vitro* data have shown that the concentration of imatinib required to inhibit cells expressing the F/P fusion by 50% (IC_{50}) is over 100 times lower than that required to inhibit cells expressing the BCR-ABL fusion observed in CML (16). As a consequence, adverse effects of imatinib, most of which are dose-dependent, have been encountered less frequently in studies evaluating effects in m-HES patients. However, some concern has been raised regarding potential toxicity of imatinib specifically associated with its use in this disorder, as severe congestive heart failure developed in three patients within days after initiation of therapy (57, 65). This may be related to massive liberation of toxic eosinophil contents in surrounding tissue following exceedingly potent and rapid destruction by imatinib. Increased serum concentrations of cardiac troponin T were observed prior to the development of congestive heart failure in two patients (65). The authors have suggested that heart function be monitored closely by echocardiograms at the beginning of therapy, and serial measurements of cardiac troponin T be performed. Rapid administration of GCs appears to be effective in handling this preoccupying complication of imatinib in m-HES patients.

Relapse during treatment with imatinib has been observed in two patients with the F/P fusion, and was shown to be associated with the appearance of a point mutation within the PDGFR α moiety of the fusion protein (16, 19). This mutation occurs in the imatinib binding pocket of the protein, thereby interfering with its ligation and conferring clinical resistance to treatment, as previously described in CML patients who relapsed under imatinib. It has recently been suggested that doses of imatinib higher than 100 mg/day may be required to achieve cytogenetic remission in m-HES, which is considered the therapeutic goal in this particular subgroup of patients by some investigators (21).

Therapeutic considerations in l-HES. Therapeutic aims in l-HES patients should include abrogating production of eosinophilopoietic cytokines by aberrant T-cells or interfering with their effects, as well as controlling expansion of these cells in hopes of preventing malignant transformation. The ability of currently used strategies to meet these two endpoints will be discussed, and future

strategies will then be proposed, based on recent studies concerning the activation pathways operating in CD3⁻CD4⁺ clones.

Glucocorticoids could theoretically meet both therapeutic aims, as they are able to inhibit production of type 2 cytokines by CD4⁺ T-cells, and interfere with clonal IL-2-dependent expansion of T-cells by reducing IL-2 production and CD25 expression *in vitro* (66). Although methylprednisolone (mPDN) displays a potent pro-apoptotic effect on CD3⁻CD4⁺ cells *in vitro* (unpublished observations), its effects on survival of the aberrant T-cell subset *in vivo* appear less clear-cut. Indeed, although GC treatment generally has little effects on the proportion of CD3⁻CD4⁺ cells, a significant decline of the clonal CD3⁻CD4⁺ subset has been reported in two cases (29, 30), and cytogenetic remission was observed in one patient with partial 6q and 10p deletions (29). Despite persistence of CD3⁻CD4⁺ cells in most patients, clinical manifestations are generally well controlled by GCs. This may be related both to the direct inhibitory effects of GCs on eosinophils themselves and to interference with cytokine production by pathogenic T-cells.

As for HU, there is currently no data indicating that it could be useful in lymphoproliferative disorders in general, and its effects on IL-5 production and expansion of pathogenic T-cells in the setting of l-HES have never been assessed *in vitro*. Although HU may indeed have a central negative effect on cytokine-driven eosinophil expansion, as indicated by normalization of eosinophil levels in one patient with CD3⁻CD4⁺ cells (34), it appears not to affect the aberrant T-cell clone.

Although most patients responding to treatment with IFN- α present case histories consistent with m-HES (61), this molecule may also have a place in the management of patients with l-HES, as it has been shown to antagonize Th2 responses, both *in vitro* and *in vivo* (67). In accordance with these studies, IFN- α decreases *in vitro* production of IL-5 by CD3⁻CD4⁺ cells isolated from patients with l-HES in a dose-dependent manner (68) and inhibits their proliferation (unpublished data). *In vivo*, administration of IFN- α to two patients in our series was rapidly followed by clinical improvement and regression of hypereosinophilia. However, these encouraging results have recently been challenged by the observation that IFN- α prolongs the survival of clonal CD3⁻CD4⁺ cells *in vitro* by inhibiting spontaneous apoptosis (40), and may therefore provide these cells with a selective advantage. Given the malignant potential of aberrant T-cells associated with l-HES, monotherapy with IFN- α should be avoided in this setting.

The recent development of anti-IL-5 mAbs, designed to target eosinophils in allergic disorders by interfering with the ligation of IL-5 to the α -chain of the IL-5R on their surface, has raised considerable interest among investigators dealing with IHES. There is strong scientific rationale for treatment of l-HES patients with anti-IL-5 mAb, as eosinophil expansion is clearly driven by exogenous (T-cell) production of IL-5. It is expected that eosinophil

levels would decline dramatically with anti-IL-5 in this variant. As for potential effects on pathogenic T-cells, it can be hypothesized that cross-talk between eosinophils and clonal Th2-like cells contributes to activation of the latter, as eosinophils are known to express B7 molecules (69) and may provide the necessary costimulatory signals for initiating autocrine IL-2-dependent T cell growth (Fig. 1). Efficacy of anti-IL-5 mAbs in controlling eosinophil levels and clinical manifestations in l-HES patients remains to be evaluated in a well-conducted clinical trial.

Recent studies investigating activation and survival requirements of CD3⁺CD4⁺ cells (52) suggest that several immunomodulatory molecules, including cyclosporine A, anti-IL-2R- α mAbs, and CTLA-4-Ig, may meet both endpoints in patients with this particular lymphocyte subset. Indeed, IL-2/IL-2R- α interactions were shown to be critical not only for the survival and proliferation of these cells, but also for their production of Th2 cytokines, and costimulatory signaling through CD28 was a prerequisite to the initiation of their autocrine IL-2-dependent activation.

Another potential therapeutic approach for patients with l-HES is extracorporeal photochemotherapy (ECP). Suppressive effects of ECP on the pathogenic T-cell clones that mediate diseases such as cutaneous T-cell lymphoma, atopic dermatitis, and graft-vs-host disease are the result of several distinct mechanisms, including induction of T-cell apoptosis and modulation of cytokine profiles in favor of type 1 responses (70). As pathogenic T-cell clones in HES circulate freely in the intravascular compartment, they could represent an ideal target for extracorporeal irradiation.

Once full-blown peripheral T-cell lymphoma has developed in patients initially presenting l-HES, classical chemotherapeutic regimens directed against lymphoid malignancy are generally administered (47). To our knowledge, there have been no reports of successful treatment of lymphoma in this setting, and all cases have been fatal. In our limited experience, chemotherapy associating cyclophosphamide, doxorubicin, vincristine, prednisone, teniposide, and bleomycin (CHVmP-BV) failed to eradicate the CD3⁺CD4⁺ T-cell clone in one patient (unpublished observations). Purine nucleoside analogs such as fludarabine and 2-chlorodeoxyadenosine have shown promising clinical activity in several indolent lymphoid malignancies, are potent suppressors of CD4⁺ T-cells, and are therefore theoretically interesting in this context (71). Indeed, these molecules are able to induce apoptosis of nondividing lymphocytes, in addition to their cytotoxic effects on proliferating cells.

Finally, complete eradication of aberrant clones associated with l-HES could be obtained by intensification of chemotherapy followed by allogeneic stem cell transplantation, as recently observed by our group in the above-

mentioned patient who was resistant to standard chemotherapy. She is still in complete remission 33 months after the procedure (unpublished observations).

Concluding remarks

On the basis of cellular and molecular investigations, it has become possible to categorize many patients initially diagnosed as IHES into well-defined pathogenic variants primitively involving either myeloid or lymphoid lineages. The profound differences in molecular pathogenesis of these variants account for their distinct clinical presentations, complications (including development of malignancy), and prognosis. Furthermore, exciting therapeutic perspectives based on underlying molecular and functional abnormalities have suddenly emerged. Imatinib mesylate has clearly become first-line therapy for m-HES patients with the F/P fusion, whereas anti-IL-5 will soon be evaluated in patients predominantly presenting features of l-HES. It follows that distinction of HES variants is critical for adequate patient management. Several potential diagnostic markers (i.e. serum tryptase for m-HES and serum TARC for l-HES) are currently under investigation in hopes of facilitating distinction between disease variants. Unfortunately, at this point in time, the primitive hematological disorder remains idiopathic in some patients, who alone are still referred to as 'IHES'. Future identification of additional molecular defects underlying variants of this syndrome is likely to consign the term 'idiopathic' HES to history, and replace it with an array of well-defined hematological disorders.

Note added in proof

Several authors have reported promising effects of anti-IL-5 mAb in patients with IHES since this review article has been submitted. Anti-IL-5 appears to be effective in controlling eosinophil levels in blood and tissues [skin (72) and esophagus (73)] as well as clinical manifestations in absence of significant adverse effects.

Acknowledgments

We would like to thank D. Zucker-Franklin for searching HTLV₁ and HTLV₂ proviral sequences in CD3⁺CD4⁺ T-cells from our patients and for serum anti-Tax antibodies. We also thank the Fonds National de la Recherche Scientifique and the Télévie program who funded much of the work related to pathogenesis of the lymphocytic variant of HES. Peter Vandenberghe has kindly accepted that we refer to clinical observations made by his group that have recently been submitted for publication.

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