

Hypereosinophilic Syndrome: Lymphoproliferative and Myeloproliferative Variants

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ABSTRACT

Idiopathic hypereosinophilic syndrome is a largely heterogeneous disorder defined as persistent, marked hypereosinophilia of unknown origin complicated by end-organ damage. Recent research in cellular and molecular biology has led to the characterization of distinct underlying hematological disorders, primitively involving cells of the myeloid or lymphoid lineage. The ability to classify many hypereosinophilic syndrome patients on the basis of pathogenesis of hypereosinophilia has radically changed therapeutic perspectives. Indeed, imatinib mesylate has become first-line therapy for patients in whom the FIP1L1-PDGFR α fusion gene is detected, whereas corticosteroids remain the mainstay for management of patients in whom hypereosinophilia is secondary to the overproduction of interleukin 5 by abnormal T-cells. Use of monoclonal anti-interleukin-5 antibodies in the latter group of patients has a strong rationale and could decrease cumulative corticosteroid doses and toxicity. As far as prognosis of these disease variants is concerned, hypereosinophilic syndrome patients with the FIP1L1-PDGFR α fusion gene may develop acute myelogenous (eosinophilic) leukemia, whereas those with clonal interleukin-5-producing T-cells have an increased risk of developing T-cell lymphoma. It is currently unclear whether timely therapeutic intervention in such patients could interfere with long-term progression toward malignant hematological disorders.

KEYWORDS: Lymphoma, IL-5, FIP1L1-PDGFR α , CD3⁺ CD4⁺ T-cell, eosinophil

Hypereosinophilia is a common biological finding, especially when mild (absolute eosinophil count < 1000/ μ L), and can be ascribed to an underlying disease in most cases. Although parasitic diseases involving tissue-invasive helminths and atopy or allergic drug reactions account for most cases, several less frequently encountered diseases may be implicated, including hematological malignancies, solid tumors, connective tissue

disorders, vasculitides, and infectious disease. In the presence of persistent hypereosinophilia, the clinician's major challenge is to make the correct diagnosis, and therapeutic options are quite straightforward. Occasionally, however, thorough evaluation fails to reveal an underlying disease, and diagnosis of organ-specific idiopathic hypereosinophilic disease (i.e., eosinophilic gastroenteritis, chronic eosinophilic pneumonia, Gleich's

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syndrome, etc.) or of idiopathic hypereosinophilic syndrome (HES) must be considered. Organ damage in such patients is directly related to the release of toxic substances by infiltrating eosinophils. In such idiopathic hypereosinophilic disorders, eosinophil levels may be markedly increased (often well beyond 1500/ μ L), and the clinician's major task is therefore to lower them, which may represent a true challenge. In this article, we revisit management of HES in light of current insight regarding pathogenic heterogeneity of this disease.

DEFINITION OF HES

The term hypereosinophilic syndrome was coined by Hardy and Anderson in 1968 in a report on three patients with persistent hypereosinophilia of undetermined etiology associated with organomegaly and cardiac involvement.¹ This was the first attempt to propose a unique diagnostic entity encompassing patients with a series of clinical manifestations arising in the context of persistently elevated eosinophil levels. In 1975, Chusid et al reviewed literature for patients with persistent idiopathic hypereosinophilia using the following inclusion criteria, which have since been widely used for diagnosis of HES: blood eosinophilia exceeding 1500/ μ L for more than 6 consecutive months, or death before 6 months, associated with signs and symptoms of hypereosinophilic disease; lack of evidence for parasitic, allergic, or other known causes of hypereosinophilia; and signs and symptoms of organ disease related to hypereosinophilia.² Application of Chusid's criteria has made it possible to describe clinical presentations and complications of HES, to define predictors of long-term prognosis, and to compare different therapeutic strategies for this disorder.

Patient series based on Chusid's definition have shown that HES predominantly affects males (male-to-female ratio 9:1) aged between 20 and 50 years.^{3,4} Major targets include the skin, heart, and nervous system, with more than 50% of patients presenting clinical complications in each of these sites. Eosinophilic infiltration of other target organs including lungs, liver, spleen, digestive system, articulations, and kidneys occurs with variable frequency. Microvascular thrombotic phenomena resulting from eosinophil-related damage to endothelium combined with activation of coagulation have been described by several groups.⁵ All published series underscore the great clinical heterogeneity and highly variable prognosis of HES, ranging from paucisymptomatic disease requiring no treatment and associated with prolonged survival to rapidly fatal disease course resulting from development of congestive heart failure or occurrence of acute leukemia. Indicators of poor prognosis have been defined by several groups, including extremely high eosinophil levels ($> 100,000$), increased serum vitamin B12, altered leukocyte alkaline phosphatase

score, presence of chromosomal abnormalities, anemia or thrombocytopenia, hepatomegaly, splenomegaly, and circulating leukocyte precursors.^{6,7} These features, all typically encountered in other myeloproliferative disorders, were associated with a more aggressive disease course characterized by poor response to systemic corticosteroids (CS) and increased likelihood of developing severe cardiac complications or acute (myelogenous or eosinophilic) leukemia.

HES would still be a hazy and unpredictable disorder if pioneers in the field had not proposed a unique definition, making it possible to group patients and analyze their outcome. However, certain aspects of Chusid's criteria have become inappropriate for patients presenting with chronic hypereosinophilia in the early 21st century. First, the duration of hypereosinophilia before diagnosis of HES can be considered has become unnecessarily long. Etiological work-up of hypereosinophilia can be accomplished faster than before, and clinicians now tend to treat patients with high eosinophil levels and potentially life-threatening organ involvement before the 6-month interval is completed. Second, major pathogenic advances have been made in the past few years, and optimal patient management is dependent on the ability to refine HES diagnosis beyond the initially proposed criteria. With the recent discovery of distinct well-characterized molecular defects ultimately leading to eosinophil expansion in patient subpopulations, the term idiopathic is actually outdated in many cases.

PATHOBIOLOGY OF HYPEREOSINOPHILIA

Accumulation of eosinophils in peripheral blood and tissues can either be the result of a primitive myeloid disorder (primary eosinophilia) or be caused by the production of eosinophilopoietic cytokines by nonmyeloid cells (secondary eosinophilia).⁸ Eosinophils are derived from myeloid progenitors (GEMM-CFU) in bone marrow through the action of three hematopoietic cytokines, GM-CSF, interleukin 3 (IL-3), and IL-5, among which only the latter is specific for eosinophil differentiation.⁹ Mature eosinophils are released into the bloodstream and rapidly migrate to peripheral tissues, namely, gut and bronchial mucosa and skin, where survival is short unless apoptosis is prevented by factors such as IL-3, IL-5, or GM-CSF.

In human pathology, dysregulated production of one or more of these cytokines by different cell populations accounts for hypereosinophilia in various disorders. Hence, malignant cells producing GM-CSF, IL-3, or IL-5 are responsible for the hypereosinophilia observed in some patients with non-Hodgkin's lymphoma, Sézary syndrome, and solid tumors such as lung cancer.^{4,10} In allergic disorders and parasitosis, the role of IL-5 in the induction of hypereosinophilia is well established.¹¹ Although several cellular sources of IL-5 have been

identified (including mast cells, basophils, and eosinophils), helper (CD4⁺) T-cells displaying a type 2 cytokine profile appear to be primarily involved in these disorders.¹² These so-called Th2 cells produce a variety of cytokines in addition to IL-5, including IL-4 and IL-13, which favor IgE synthesis. Th2-mediated immune responses are thus characterized by the association of hypereosinophilia, high serum IgE levels, and eventually polyclonal hypergammaglobulinemia.

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, proinflammatory cytokines, and lipid mediators.⁹ There is no clear correlation between eosinophil levels and severity of organ involvement in patients with HES. Some patients with isolated cutaneous manifestations remain untreated over a prolonged time period and never develop cardiac complications despite marked hypereosinophilia, whereas others will rapidly develop severe subendocardial fibrosis in the presence of lower eosinophil levels. There is an obvious need for markers of eosinophil "aggressiveness" that could predict clinical outcome, but identification of such markers has proven difficult until now. Eosinophil hypodensity reflects eosinophil degranulation but fails to correlate with disease activity. Serum levels of the eosinophil-derived cationic protein MBP correlate with eosinophil levels in hypereosinophilic patients¹³ but have not yet been shown to be indicators of disease activity. Expression levels of candidate activation markers on eosinophils including CD69 or CD25 remain to be assessed in relation to disease severity.

PATHOGENICALLY DISTINCT VARIANTS OF HES

The striking clinical heterogeneity among patients with HES and the occasional development of malignancy involving either the myeloid or the lymphoid lineage strongly indicate 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 HES.

The Myeloproliferative Variant of HES

Early investigators singled out a subgroup of patients with clinical and biological features reminiscent of chronic myelogenous leukemia (CML) and other myeloproliferative syndromes as presenting a more aggressive disease variant, qualified as the myeloproliferative variant of HES (m-HES).^{2,6,7} However, existence of a true myeloproliferative disorder remained purely speculative at the time, as demonstration of eosinophil/

myeloid clonality was rarely feasible. A few reports did provide support for a primitive myeloid disorder by showing chromosomal abnormalities adjacent to eosinophil granules¹⁴ or skewed methylation patterns of X-linked genes in purified granulocytes or eosinophils.^{15,16} Unfortunately, applicability of these methods in HES is limited, as abnormal karyotypes 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, one group has succeeded in providing cytogenetic evidence for a clonal myeloproliferative disorder in a significant proportion (currently estimated between one-fourth and one-third) of HES patients through the description of an interstitial deletion on chromosome 4q12, resulting in fusion of FIP1L1 and PDGFR α genes.¹⁷ The fusion gene is in-frame and encodes a FIP1LI-PDGFR α (F/P) protein with constitutive tyrosine kinase activity, which is highly susceptible to the tyrosine kinase inhibitor, imatinib mesylate. Similar findings have been confirmed by other groups both on patient samples^{18,19} and in EOL-1 cell lines derived from a patient who developed acute eosinophilic leukemia following diagnosis of HES.²⁰ The central role of this fusion gene in disease pathogenesis is supported by its disappearance in most patients successfully treated with imatinib.²¹ Furthermore, its transforming potential is indicated by its ability to render the murine hematopoietic cell line Ba/F3 IL-3-independent in vitro and by its presence in the EOL-1 cell line.^{17,20}

In addition to the F/P fusion, it is likely that other cytogenetically distinct primitive myeloid disorders are present in some HES patients. Approximately two-thirds of patients responding to imatinib mesylate present the F/P fusion gene,¹⁷ indicating involvement of other as-yet-unidentified imatinib-sensitive tyrosine kinases in the remaining third. In yet other patients, eosinophil expansion may be dependent on unrelated molecular defects that are not inhibited by imatinib mesylate, such as mutations involving growth receptors or intracellular signaling pathways.

Whether "m-HES" patients in whom such cytogenetic abnormalities are detected should still be considered as presenting HES at all is debatable, and the more appropriate diagnosis of chronic eosinophilic leukemia has been recommended for those in whom eosinophil clonality, clonal cytogenetic abnormalities within cells of the eosinophil lineage (inclusive of the F/P fusion), or increased blasts can be demonstrated.^{8,22} The term m-HES could then be used for patients with presumptive, but not conclusive, evidence of an underlying primitive myeloproliferative disorder. Klion et al²¹ considered that the diagnosis of m-HES was appropriate when four of the following criteria were fulfilled: presence of dysplastic eosinophils, increased serum B12, increased serum tryptase, anemia/thrombocytopenia,

hepatosplenomegaly, increased bone marrow cellularity with an increased proportion of immature cells, myelofibrosis, and dysplastic mast cells in bone marrow. In addition, it is likely that F/P fusion-negative patients that respond to imatinib present m-HES. For the purpose of this article, we continue to use the term m-HES for all patients with an underlying myeloid disorder, whether clonality is demonstrated or not.

Lymphocytic Variant of HES

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,^{23,24} led authors to suggest that T-cells could be involved in disease pathogenesis through the release of soluble factors. Emergence of the Th1/Th2 paradigm rekindled interest in the pathogenic role of T-cells in HES, as the association of hypereosinophilia with increased IgE levels in some patients indicated the possibility of a Th2-mediated 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.²⁵ Since then, IL-5-producing T-cell subsets have been described in blood of about 40 patients with HES,^{26,27} and it can be estimated that approximately one-third of patients fulfilling HES criteria have an underlying primitive T-cell disorder.²⁸ The allegedly pathogenic T-cells generally display an aberrant surface phenotype, and CD3⁺CD4⁺ cells represent the most frequently encountered subset in this setting. A CD3⁺CD4⁺CD8⁺ phenotype has been described in four patients. The aberrant lymphocyte subsets generally express surface antigens characteristic of activated (HLA-DR⁺ or CD25⁺) memory (CD45RO⁺) T-cells,^{26,29} and CD3⁺CD4⁺ cells often display high-level expression of CD5 and frequent loss of surface CD7 or CD27 expression.²⁹ Phenotypically aberrant T-cells are clonal in many cases, and chromosomal abnormalities, including 16q breakage, partial 6q or 10p deletions, and trisomy 7, have been reported.²⁷ Their ability to produce IL-5 accounts for induction of hypereosinophilia. Furthermore, CD3⁺CD4⁺ cells have been shown to produce the Th2 cytokines IL-4 and IL-13, as well as IL-2, TNF- α , GM-CSF, and even the Th1 cytokine IFN- γ in some cases. On the basis of currently available data, the "lymphocytic variant" of HES (l-HES) can be defined as a primitive lymphoid disorder characterized by non-malignant expansion of a T-cell population able to produce eosinophilopoietic cytokines (generally IL-5).

The primary events leading to clonal T-cell expansion may differ among hypereosinophilic patients

with distinct phenotypic abnormalities. Simon et al have shown that aberrant T-cells lacked CD95/Fas-R expression in 8 of 16 patients of their series²⁶ and demonstrated that deficient Fas-mediated apoptosis was involved in the expansion of a CD3⁺CD4⁺CD8⁺ IL-5-producing lymphocyte subset in one such patient.³⁰ In contrast, CD3⁺CD4⁺ cells from our patients express Fas-R and are highly sensitive to apoptosis following exposure to soluble Fas-ligand *in vitro*.³¹ It is likely that absent membrane expression of the TCR/CD3 complex by this particular subset of T-cells is involved in its uncontrolled expansion (see section on prognosis of HES). As for the events leading to loss of this T-cell receptor complex, a common pathogenic agent may be responsible. It has recently been shown that CD3⁺CD4⁺ cells from one HES patient specifically display defective transcription of the CD3- γ chain, whereas transcription of the other chains belonging to the TCR/CD3 complex is maintained.³² Thus, although intact CD3- ϵ and TCR- α/β chains are detectable in the intracellular compartment, membrane translocation of the complete TCR/CD3 complex, which is dependent on the expression of CD3- γ , does not occur. Interestingly, HTLV₁ is known to target CD3- γ chain transcription, leading to absent or low expression of the TCR/CD3 complex by infected CD4⁺ T-cells.³³ We have failed to detect evidence for HTLV₁ infection in all hypereosinophilic patients with CD3⁺CD4⁺ T-cells except one, who presented positive serology and developed T-cell lymphoma. We believe that another closely related pathogen could be involved in the pathogenesis of CD3⁺CD4⁺ T-cell-associated HES.

CLINICAL PRESENTATIONS OF HES

Clinical presentations of HES are notoriously variable and unpredictable, as previously underlined. With increasing knowledge regarding pathogenesis of disease variants, however, more homogenous patient groups can be identified.

Clinical Profile of Patients with m-HES

Depending on the molecular abnormality underlying eosinophil expansion in m-HES patients, clinical presentations may be variable. Indeed, two patients in whom eosinophil clonality was demonstrated on the basis of X-linked polymorphisms had a distinct clinical profile (absence of myeloproliferative features, increased serum IgE levels, and striking response to CS therapy)^{15,16} compared with patients in whom the F/P fusion is detected by FISH (fluorescence *in situ* hybridization) or reverse transcriptase polymerase chain reaction (RT-PCR). The female patients in whom X-linked polymorphisms were used for detection

of clonality were unlikely to have the F/P fusion gene, as this molecular defect is almost exclusively found in male patients.

Clinical and biological features frequently encountered in patients with the F/P fusion gene include anemia or thrombocytopenia, increased serum B12 levels, mucosal ulcerations, endomyocardial fibrosis, and splenomegaly.^{18,19} It has been suggested that mast cells may be implicated in the increased frequency of fibrotic complications observed in these patients.^{18,21} Bone marrow biopsies have indeed shown increased numbers of atypical spindle-shaped mast cells and associated myelofibrosis in patients with increased serum tryptase levels and the presence of the F/P fusion. It is likely that the atypical mast cells are part of the F/P fusion-positive clone, as they disappear in patients who respond to imatinib. Although mechanisms whereby mast cells contribute to development of fibrosis remain largely unknown, mast cell-derived tryptase could serve as a mediator between clonal eosinophils and fibroblasts.

Clinical Profile of Patients with I-HES

In contrast to m-HES, I-HES affects females at least as much as males. A previous history of typical atopic disease is frequently encountered. Cutaneous manifestations, including pruritus, eczema, erythroderma, urticaria, and angioedema, are observed in virtually all patients reported in the literature.^{26,27,29} Other complications of hypereosinophilia in patients with I-HES occur most commonly in the lungs and the digestive system, whereas very few patients develop endomyocardial fibrosis despite high eosinophil levels.

Skin biopsies in patients with aberrant T-cell subsets and cutaneous manifestations show prominent eosinophil accumulation. Skin-infiltrating T-cells were shown to produce IL-5 in one study.³⁴ However, it is currently unknown whether expression of specific adhesion molecules or chemokine receptors accounts for preferential migration of aberrant T-cell subsets to skin. CD3⁺CD4⁺ cells do not express cutaneous lymphocyte antigen (unpublished observations), but they consistently lack surface CD7, a characteristic of skin-homing T-cells.³⁵ Interestingly, the principal targets in this HES variant (i.e., skin, lungs, digestive tract) are 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 (increased IgM and/or IgG levels) may be observed.²⁷ Finally, among hypereosinophilic patients with an IL-5-producing CD3⁺CD4⁺ T-cell clone, some present a clinical profile indistinguishable from that encountered in Gleich's syndrome, or episodic angioedema with eosinophilia, a disease characterized

by spontaneously remitting episodes of angioedema, high serum IgM levels, and hypereosinophilia.^{36,37}

Lung Involvement in the HES

Signs and symptoms associated with lung involvement are present in about 40% of HES patients.^{3,38} Clinical presentations are variable and probably reflect different functional characteristics of the infiltrating eosinophils. In the majority of cases, patients present with chronic dry cough in the absence of radiological or pulmonary function test abnormalities. In others, pleural effusion or pulmonary infiltrates are observed. These may be diffuse or focal and affect any region of the lungs.⁴ Eosinophils are found in BAL fluid and lung biopsies, and fibrosis may develop. Functional consequences of pulmonary infiltrates range from restrictive or obstructive lung disease to ARDS (adult respiratory distress syndrome), requiring mechanical ventilation.³⁹

The association of peripheral hypereosinophilia and lung infiltrates with eosinophils in BAL fluid or biopsies can be observed in other eosinophil-mediated pulmonary disorders such as acute eosinophilic pneumonia (EP), chronic EP, allergic bronchopulmonary aspergillosis, drug-induced EP, Churg-Strauss syndrome, and parasitic EP (see recent review⁴⁰). These disorders can be distinguished from HES in most cases on the basis of clinical and biological features, disease course, and high-resolution computed tomography findings. On occasion, isolated lung involvement is the presenting manifestation of HES, making differential diagnosis with chronic (or acute) EP difficult until disease expression occurs in another target tissue.

Since the recent description of F/P fusion-associated disease and IL-5-producing T-cell-mediated HES, there has been little information made available on the nature of lung involvement in these variants. Interestingly, Klion et al have shown that among nine HES patients with elevated serum tryptase levels (most of which were shown to have the F/P fusion gene), all four patients with lung involvement had restrictive lung disease, whereas among six patients with low serum tryptase levels (none of which had the F/P fusion gene), three had obstructive lung disease.¹⁸ This observation indicates once again that F/P fusion-associated disease is complicated by fibrosis of target organs. In their review of 16 patients with aberrant T-cell populations, Simon et al observed lung involvement in two cases, one of which had clear T-cell clonality, but the obstructive versus restrictive nature of lung disease was not specified.²⁶

PROGNOSIS OF HES

Prognosis of HES as a whole is dependent on the severity of end-organ damage, especially heart involvement, and on the development of malignancy. Survival

rates have increased dramatically since Chusid's initial 1975 retrospective study as a result of improved prevention and management of target organ damage (i.e., rapid lowering of eosinophil levels and better surgical techniques for cardiac complications), improving from a 3-year survival of 12% in Chusid's study to a 10-year survival of 70% in Fauci's prospective study published in 1982. However, occurrence of malignant hematological disorders remains a subject of concern.

The Myeloproliferative Variant

Endomyocardial fibrosis is frequently observed in patients with F/P-associated HES and is largely irreversible,^{19,21} as shown by persistence of clinical manifestations and echocardiographic abnormalities in most patients whose eosinophil levels and other complications are well controlled by imatinib mesylate. 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 in whom rapid and efficient control of eosinophil levels is essential. 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 the occurrence of disease-related death.¹⁸

As for the development of a malignant disorder, follow-up of patients previously classified as m-HES based on their clinical presentation has shown that some will experience acute blastic transformation (i.e., acute myeloid leukemia or granulocytic sarcoma).⁸ At least two patients with the F/P mutation have been reported to develop leukemia,^{19,20} in accordance with the transforming potential of the F/P fusion *in vitro*.

As a whole, the 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 resulting from both development of cardiac complications and increased risk of developing acute myeloid leukemia. It can be hoped that timely administration of imatinib to these patients will modify disease course and delay or prevent malignant transformation.

The Lymphocytic Variant

HES patients with an aberrant IL-5-producing T-cell population rarely experience severe or life-threatening end-organ damage (in particular, heart involvement) and have better short-term prognosis compared with those with m-HES. However, long-term prognosis is affected by the possible occurrence of T-cell malignancy years after diagnosis of HES. Peripheral T-cell lymphoma arising in this patient subgroup (reviewed in⁴¹) may conserve the initially observed aberrant phenotype,

indicating that the pathogenic T-cell population is pre-malignant. Repeated studies of 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).⁴² Similar deletions in other malignancies (including T-cell and B-cell disorders,^{43,44} as well as solid tumors) indicate that this region is likely to harbor tumor suppressor genes.

Only a proportion of patients with l-HES progress to T-cell lymphoma, and to date there are no clear-cut predictors of malignant outcome. Loss of surface TCR/CD3 expression by CD4⁺ cells has been described in other disorders associated with an increased incidence of T-cell lymphoma, namely, ataxia-telangiectasia⁴⁵ and angioimmunoblastic lymphadenopathy,⁴⁶ indicating that this particular phenotype may predispose T-cells to malignant transformation. It can be hypothesized that as a result of absent TCR/CD3 expression, these CD4⁺ T-cells are exposed to alternative activation pathways that may be less tightly linked with negative regulatory mechanisms, ultimately leading to uncontrolled clonal expansion. It has been shown that antigen-presenting cells are able to induce proliferation of CD3⁺CD4⁺ cells *in vitro* through engagement of CD2 and CD28 surface molecules and initiation of an autocrine and probably paracrine IL-2/IL-2R- α loop.⁴⁷

The evidence for an increased risk of lymphoid malignancy in patients with l-HES challenges the long-held notion that isolated cutaneous manifestations associated with serum hyperIgE in the setting of the HES are markers of good prognosis. Moreover, identification of clonal CD3⁺CD4⁺ cells in blood of three patients with Gleich's disease^{27,36,37} indicates that this reputedly benign idiopathic hypereosinophilic disorder may also be associated with an increased risk of malignant transformation.

MANAGEMENT OF PATIENTS WITH HES

Management of HES patients remains a challenge and should be organized in collaboration with experienced physicians and departments. Indeed, correct pathogenic classification of a patient, which is critical for the choice of treatment, is dependent on techniques that are not available on a routine basis. Moreover, modern therapeutic strategies are based on use of molecules that are not yet widely distributed among practitioners but, rather, are assessed in clinical trials.

Diagnosis of HES Variants

Distinction of an underlying myeloid versus lymphocytic disorder is a critical first step in establishing optimal

guidelines for follow-up and treatment. We have seen that predominant cutaneous manifestations in the absence of heart involvement, associated with serum hyper-IgE or polyclonal hypergammaglobulinemia, should arouse suspicion of l-HES. Likewise, splenomegaly, heart involvement, increased vitamin B12 levels, anemia or thrombocytopenia, and the presence of immature myeloid precursors in peripheral blood are indicative of m-HES. However, these easily assessed features are not entirely specific for a given disease variant, and further testing including molecular characterization remains essential for pathogenic classification of patients. Investigations that should be performed are shown in Table 1 and have been commented in a recent review.²⁷

Search for the F/P fusion by RT-PCR or FISH should be performed in all patients with HES, as its presence guides therapy with the tyrosine kinase inhibitor, imatinib mesylate. Deletion of the CHIC2 locus, situated between FIP1L1 and PDGFR α on chromosome 4q, could serve as a surrogate marker for the fusion.⁴⁸

Identification of patients with l-HES is based on analysis of circulating and bone marrow-derived T-cells, including lymphocyte phenotyping in search of

a phenotypically aberrant T-cell subset (principally CD3⁻CD4⁺ or CD3⁺CD4⁻CD8⁻), analysis of TCR gene rearrangement patterns using both Southern Blot (for TCR β) and PCR amplification (for TCR γ) in search of T-cell clonality, and analysis of cytokine profiles. Surface antigens that should be assessed by flow cytometry in addition to CD3, CD4, and CD8 include CD2, CD5, CD6, CD7, CD25, CD27, CD45RO, and HLA-DR, as phenotypic abnormalities are occasionally limited to alterations of staining intensity of these markers on a T-cell subset.²⁶ The fact that T-cell clonality is not detected in all patients with a phenotypically aberrant lymphocyte subset may reflect true absence of clonality, or clonality may be undetected because of clonal deletion of TCR chain genes,⁴⁹ choice of primers in PCRs, or lack of sensitivity when aberrant cells represent a small proportion of total lymphocytes. Purification of T-cell subsets may be required to reveal a clonal TCR rearrangement pattern in some cases.²⁹ Finally, enhanced production of eosinophilopoietic (IL-3, GM-CSF) or type 2 (IL-4, IL-5, IL-13) cytokines by T-cells can be demonstrated by measuring cytokine concentrations in supernatants of cultured PBMC (peripheral blood mononuclear cells) or purified

Table 1 Investigations Required for Pathogenic Classification of HES

Available in most hospital settings

- Complete blood count with leukocyte differential
- Microscopic examination of peripheral blood smear
- Serum IgE
- Serum IgG, IgA, IgM
- Serum Vitamin B12
- Leukocyte alkaline phosphatase score
- Bone marrow smear and biopsy (with staining for tryptase and reticulin)
- Lymphocyte phenotyping
- TCR gene rearrangement analysis (Southern blot and polymerase chain reaction)
- Conventional cytogenetic analysis on peripheral blood and bone marrow
- Abdominal ultrasound (measurement of liver and spleen)
- Echocardiogram and cardiac magnetic resonance imaging when possible

Investigations referred to qualified laboratories

- Serum tryptase
- Serum TARC (thymus and activation-regulated chemokine)
- FIP1L1-PDGFR α fusion
RT-PCR, FISH
- 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 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
intracellular cytokines at the single cell level by flow cytometry
cytokines in PBMC culture supernatants

*When found normal in routine investigations.

aberrant lymphocyte subsets 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 HES, as certain abnormalities, including t(5;12)(q33;q13) or t(8;13), may actually change diagnosis to chronic eosinophilic leukemia.²² Special attention should be paid to choice of mitogens for karyotyping aberrant T-cell clones, as CD3⁺CD4⁺ and CD3⁺CD4⁺CD8⁺ cells respond poorly to stimulation with PHA, and mitosis obtained in these conditions is more likely to stem from normal T-cells. Strikingly, most reported chromosomal abnormalities in patients with l-HES have been observed following use of IL-2 as a mitogen.^{29,50,51} Hence, as previously recommended for Sézary cells,⁴⁴ growth-promoting cytokines such as IL-2 or IL-7 should be added to cultures before cytogenetic analysis of aberrant T-cells.

Bone marrow assessment should include both a smear and a biopsy. Only the latter may reveal the existence of underlying lymphoma in a patient with chronic hypereosinophilia, and staining for tryptase and reticulin may reveal the presence of dysplastic spindle-shaped mast cells and myelofibrosis in patients with F/P-associated m-HES.¹⁸

In some instances, thorough evaluation using all the procedures enumerated in Table 1 fails to reveal clear-cut existence of a primitive myeloid or lymphoid disorder. Patients in this truly "idiopathic" HES group should be stratified as more or less likely to present myeloid versus lymphoid disease, based on clinical and biological features (see Fig. 1), and be treated accordingly.

Identification of diagnostic markers consistently associated with either myeloid or lymphoid expansion could facilitate categorization of patients in this third "idiopathic" group. Serum tryptase levels are increased (> 11.5 ng/ml) in patients with F/P-associated m-HES and are not influenced by administration of GCs, HU, cyclosporine A, or IFN- α .¹⁸ Whether 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, the chemokine TARC has gained interest as a potential marker of Th2-mediated disorders, and measurement of serum levels in HES patients may prove to be useful for diagnosis of this variant. Serum TARC levels were 10–100-fold higher in 13 patients with l-HES compared with four HES patients with no evidence for an underlying lymphoid disorder, whose levels were similar to healthy control subjects.⁵² Unfortunately, serum TARC levels decrease rapidly following initiation of corticosteroids. Other potential markers of lymphoproliferative or Th2 disorders evaluated so far have failed to correlate with the

presence of IL-5-producing T-cell subsets in patients with HES. Increased serum IgE is neither sensitive nor specific for this variant; approximately three-quarters of patients with clonal CD3⁺CD4⁺ cells reported in the literature have high serum IgE levels,²⁷ but increased IgE has also been observed in one patient with the F/P fusion²⁰ and in two patients in whom eosinophil clonality was demonstrated by nonrandom X chromosome inactivation.^{15,16} Neither serum IL-5 levels nor sCD25 levels reflect T-cell-mediated disease, as they may be increased in both l-HES and m-HES patients.²⁹ A likely explanation is that activated eosinophils themselves, in addition to T-cells, are able to produce and secrete these molecules.

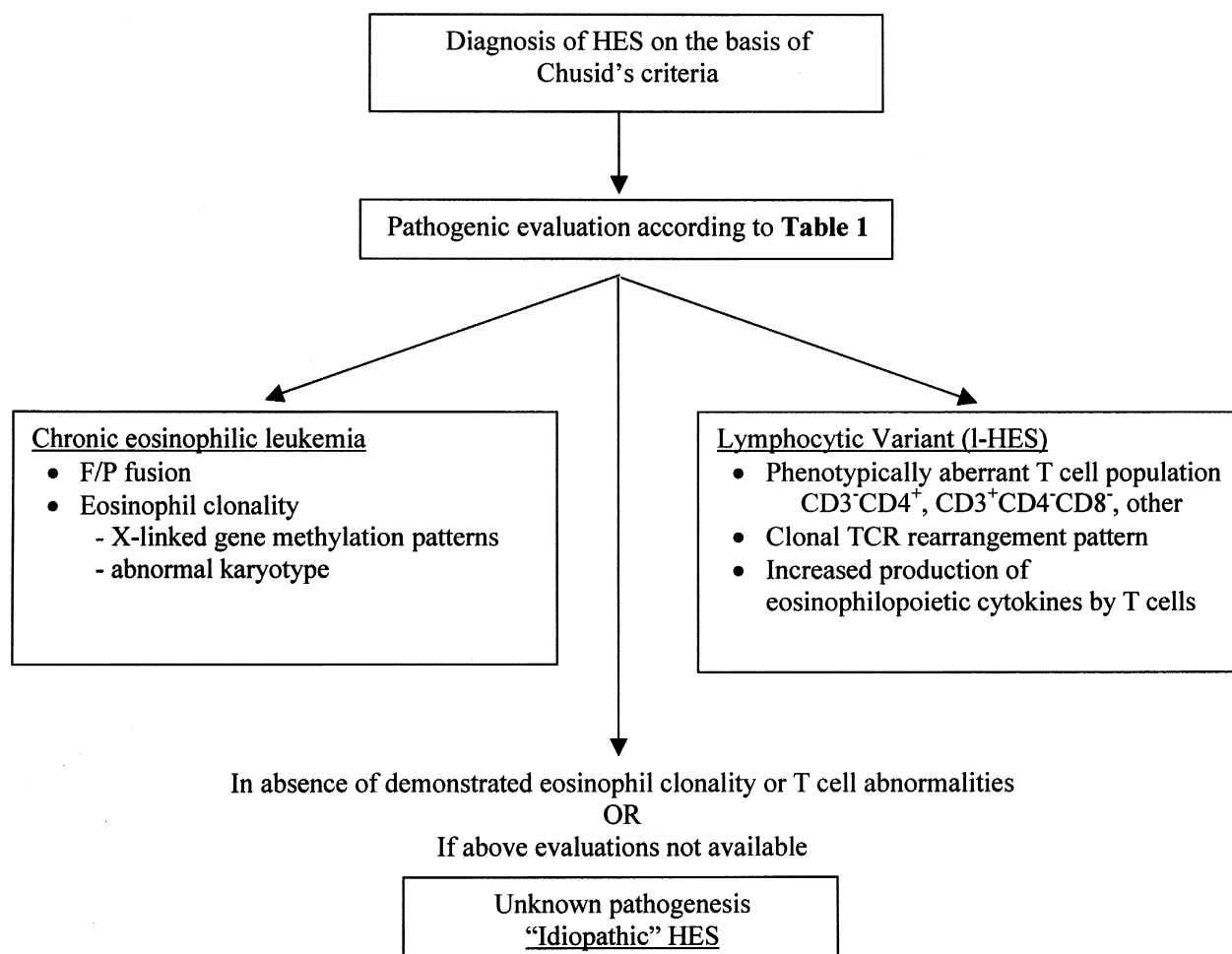
Therapeutic Perspectives for HES

Therapeutic strategies designed to control eosinophil levels in patients with HES were defined in 1975 and have relied heavily on use of corticosteroids (CSs) and hydroxyurea (HU). Most agents used to treat HES have been chosen on the basis of the supposedly myelogenous nature of this disorder and have paralleled those proposed for CML. IFN- α was introduced in the early 1990s on the basis of encouraging observations and has proven to be the most useful alternative,⁵³ whereas several additional cytostatic agents have produced overall less satisfying results. With the recent description of a chromosomal rearrangement resulting in creation of a fusion gene with constitutive tyrosine kinase activity in some patients, and demonstration of an underlying lymphocytic disorder in others, therapeutic perspectives have changed radically in the past few years.

Therapeutic Considerations in m-HES

As expected for myeloproliferative 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 and vincristine. For patients with increased blasts or other signs of malignant progression, stem cell transplantation has proven effective.

More recently, imatinib mesylate, a tyrosine kinase inhibitor used for treatment of CML patients with the bcr-abl translocation,⁵⁴ has proven highly effective in a subgroup of HES patients,^{17,21,55} many of which (approximately two-thirds) present the F/P mutation. Imatinib has been shown both to inhibit phosphorylation of the F/P protein and to induce apoptosis of cells expressing this protein.²⁰ 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 in most,^{19,21} but not all,⁵⁶ cases. Reversal of

**Presumed myeloid disorder**

- Increased serum B12
- Splenomegaly
- Anemia, thrombocytopenia
- Increased circulating myeloid precursors
- Dysplastic eosinophils
- Increased serum tryptase
- Spindle-shaped mast cells in bone marrow
- Myelofibrosis
- Significant end-organ damage (heart, nervous system)
- Imatinib responsive

Presumed lymphocytic disorder

- Increased serum IgE
- Increased serum IgGAM
- Predominant cutaneous manifestations
- Absence of heart involvement
- Good response to glucocorticoid therapy
- History of atopy
- Increased serum TARC

Figure 1 Classification of hypereosinophilic syndrome patients.

bone marrow pathology (i.e., disappearance of atypical spindle-shaped mast cells and decreased reticulin staining in patients with myelofibrosis) and normalization of serum tryptase levels have been reported.²¹ Cytogenetic remission can be achieved in most patients with the F/P fusion, provided the dose of imatinib is adapted to this endpoint. Given the scientific rationale for treatment of F/P-positive patients with imatinib, and the striking

rapidity and potency of its effects in clinical practice, this molecule has rapidly become a first-line treatment for these patients.

m-HES patients generally require lower doses of imatinib (100 mg/day) to induce clinical remission than do patients with CML, because the concentration of imatinib required to inhibit cells expressing the F/P fusion by 50% (IC₅₀) is over 100 times lower than that

required to inhibit cells expressing the bcr-abl fusion.¹⁷ However, some clinicians advocate use of higher doses in HES patients (400 mg/day) to ensure cytogenetic remission and prevent acquired resistance to treatment. Indeed, relapse under imatinib has been observed in patients with the F/P fusion and is associated with the appearance of a point mutation within the PDGFR α moiety of the fusion protein interfering with ligation of imatinib, as previously described in patients with CML.¹⁷ Development of other tyrosine kinase inhibitors that would effectively inhibit F/P protein kinase activity has become a major goal for investigators, and several alternative molecules have already been identified, one of which appears to inhibit imatinib-resistant cells.⁵⁷

Toxicity of imatinib specifically observed in HES patients may occur in the presence of preexisting (but not necessarily clinically overt) cardiac involvement and is likely related to local destruction of eosinophils by imatinib and massive release of toxic substances. Occurrence of severe congestive heart failure, preceded by increased serum levels of troponin T, has been reported in a few patients within days after initiation of therapy.^{58,59} Heart function should be monitored closely, and serial measurements of troponin T should be performed at the beginning of therapy. Rapid administration of CSs appears to be effective in handling this preoccupying complication.

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 the hope of preventing malignant transformation.

CSs could theoretically meet both therapeutic aims, as they 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*.⁶⁰ Although CSs are very successful in decreasing eosinophil levels in patients with l-HES, and therefore in controlling clinical manifestations, effects on pathogenic T-cells are variable. In most cases, the proportion of CD3⁺CD4⁺ cells is not affected by CSs; however, a significant decline of this population has been reported in two cases.^{29,49}

As for HU, there are 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 the normalization of eosinophil levels in one patient with CD3⁺CD4⁺ cells,⁶¹ it appears not to affect the aberrant T-cell clone.

Although most reported patients responding to treatment with IFN- α present case histories consistent with m-HES, this molecule may also have a place in management of patients with l-HES, as it has been shown to antagonize Th2 responses, both *in vitro* and *in vivo*. More specifically, IFN- α decreases *in vitro* production of IL-5 by CD3⁺CD4⁺ cells isolated from patients with l-HES in a dose-dependent manner⁶² and inhibits their proliferation. However, IFN- α prolongs survival of clonal CD3⁺CD4⁺ cells *in vitro* by inhibiting spontaneous apoptosis³¹ 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, and we would advocate its use in association with a therapeutic agent displaying proapoptotic activity on the clonal T-cell population, such as CS (unpublished data).

Monoclonal anti-IL-5 mAbs have recently been designed to target eosinophils by interfering with ligation of IL-5 to the α -chain of the IL-5R on their surface.⁶³ There is a 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. Encouraging results have been reported in two studies, in which hypereosinophilic patients with cutaneous and digestive involvement showed a rapid decline of blood and tissue eosinophil levels shortly after intravenous administration of anti-IL-5,^{34,64} associated with improvement in clinical manifestations, allowing them to taper CS treatment. Use of anti-IL-5 mAbs could also be extended to patients with m-HES, as indicated by its efficacy in one patient who tested positively for the F/P fusion.⁶⁵ Although anti-IL-5 mAbs have been shown to display an excellent safety profile, some concern regarding rebound hypereosinophilia during therapy has been raised. One study using anti-IL-5 at low doses has provided evidence that serum IL-5 levels actually increase during treatment,⁶⁶ but this evidence remains to be confirmed with the currently proposed regimens using higher doses.

Another 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.⁶⁷ Whether alemtuzumab is responsible for an increased incidence of cardiac dysfunction in patients with Sézary syndrome, which could be relevant for treatment of HES patients, is a subject of debate.⁶⁸

Future clinical trials for patients with l-HES could explore different immuno-modulatory agents or strategies, including cyclosporine A, anti-IL-2R- α mAbs, and extracorporeal photochemotherapy. Nuclear

extracts from CD3⁺CD4⁺ cells have been shown to contain high levels of NF-ATc2,³² a major downstream target of cyclosporine A, which could be involved in decreased transcription of CD3- γ chain gene and enhanced transcription of type 2 cytokine genes. Furthermore, IL-2/IL-2R- α interactions were shown to be critical for survival and proliferation of CD3⁺CD4⁺ cells, as well as for production of Th2 cytokines,⁴⁷ providing rationale for both cyclosporine A and anti-IL-2R- α mAbs. As for extracorporeal photochemotherapy, induction of T-cell apoptosis and modulation of cytokine profiles in favor of type 1 responses account for suppressive effects on pathogenic T-cells associated with cutaneous T-cell lymphoma, atopic dermatitis, and graft versus host disease.⁶⁹ As pathogenic T-cell clones in HES are found in peripheral blood, they could represent an ideal target for extracorporeal irradiation.

Once full-blown peripheral T-cell lymphoma has developed in patients initially presenting l-HES, eradication of malignant T-cells is not easily achieved using classical chemotherapeutic regimens. Purine nucleoside analogues such as fludarabine and 2-chlorodeoxyadenosine appear promising in indolent lymphoid malignancies, are potent suppressors of CD4⁺ T-cells, and are therefore theoretically interesting in this context.⁷⁰ Finally, complete eradication of aberrant clones associated with l-HES could be obtained by intensification of chemotherapy followed by allogeneic stem cell transplantation, as observed by our group in one patient with CD3⁺CD4⁺ cells who was resistant to standard chemotherapy. She is still in complete remission 42 months after the procedure.

CONCLUDING REMARKS

On the basis of cellular and molecular investigations, it has become possible to classify many patients initially diagnosed with HES into well-defined pathogenic variants primitively involving either myeloid or lymphoid cells. 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. Global immuno-suppression using classical therapeutic agents is no longer necessary to control hypereosinophilia in all patients, as it has become possible to individualize treatment by specifically targeting the molecules that drive disease.

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