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Hypereosinophilic syndrome variants: diagnostic and therapeutic considerations

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Hypereosinophilic syndromes (HES) are a group of disorders characterized by persistent and marked hypereosinophilia (>1500 per microliter) not due to an underlying disease known to cause eosinophil expansion (such as an allergic drug reaction or parasitic infection), and which is directly implicated in damage or dysfunction of at least one target organ or tissue.[1,2] Although rare, HES have recently nurtured much interest, as fascinating pathogenic mechanisms have been discovered in patient subgroups, and novel targeted therapeutic approaches have recently been proven efficacious. Efforts are now being directed towards improving diagnostic criteria and classification of disease forms,[2] in order to better reflect these advances, and more importantly to provide physicians with a practical diagnostic approach to patients in whom chronic damage-inducing hypereosinophilia can not be resolved by treating an easily recognized underlying cause. However, this is challenging, as pathogenesis remains unknown in the majority of patients, and there are currently no valid biomarkers which reflect underlying mechanisms leading to hypereosinophilia. Agreement on definitions is also paramount to design prospective observational studies on large multi-center patient cohorts, aiming to better define natural disease course and to identify markers of disease activity and prognosis. The ultimate goal of these endeavors is the optimization of treatment recommendations, targeting underlying molecular mechanisms when possible, and, for the majority of remaining patients, taking into account the heterogeneity of clinical profiles and disease severity so that therapeutic and disease aggressiveness are best matched.

Well-characterized pathogenic mechanisms leading to hypereosinophilia described so far in patients fulfilling classical HES diagnostic criteria involve: (i) stem cell mutations leading to expression of PDGFRA-containing fusion genes with constitutive tyrosine kinase (TK) activity (mainly the FIP1L1-PDGFRA fusion gene), and (ii) sustained overproduction of IL-5 by activated T-cell subsets with unusual phenotypes and/or clonal TCR gene rearrangement patterns (Table 1).

Discovery of the cryptic interstitial deletion on chromosome 4q12, leading to the fusion of FIP1L1 and PDGFRA genes, has represented a major breakthrough in that patients harboring this mutation respond extremely well to treatment with low doses of the TK inhibitor, imatinib mesylate (Glivec).[3] This discovery was made following the empirical observation that 4 out of 5 patients with HES responded well to Glivec.[4] Use of agents known to be effective in chronic myeloid leukemia for treatment of HES has been a classical strategy since initial description of this syndrome, given the widely held notion that HES could be a chronic myeloproliferative disorder, at least in some patients with features including hepato- and/or spleno-megaly, increased vitamin B12, anemia, thrombocytopenia, and circulating myeloid precursors. The dramatic reponse to Glivec in these 4 patients suggested that eosinophil expansion was driven by deregulated activity of one of the imatinib-responsive TK, a hypothesis that was proven correct shortly thereafter by Cools *et al.*[3] and Griffin *et al.*[5] in patients with HES, and in the Eol-1 cell line derived from a patient with HES, respectively. Although patients with this mutation are more adequately classified as chronic eosinophilic leukemia (CEL) given the clonal nature of eosinophil expansion, the cells are morphologically

indistinguishable from normal mature eosinophils, and karyotypes are normal in the large majority of cases; patients therefore present clinically as typical HES cases.

The FIP1L1-PDGFRA fusion has been detected in highly variable proportions of patients with chronic unexplained hypereosinophilia, probably due to referral bias of patients towards different clinical sub-specialities according to predominant disease manifestations. In a recent retrospective study on a large cohort of patients fulfilling classical diagnostic criteria for HES from 11 different centers, 11% of patients were shown to harbor this fusion gene (Ogbogu *et al.*, submitted for publication). Another large, combined retrospective and prospective study on patients with absolute eosinophil levels above 1500 per microliter consecutively recruited in a single center reported a 14% prevalence of this mutation.[6]

Early detection of FIP1L1-PDGFRA in patients with chronic unexplained hypereosiniphilia is now considered critical for optimal management, as its presence is associated with high spontaneous morbidity and mortality rates,[7] and imatinib represents an extremely efficacious and generally well-tolerated first-line therapeutic agent for this condition. Thus, PCR for fusion gene detection and FISH for demonstration of CHIC2 deletion are becoming increasingly available in many specialized centers. Given the poor prognosis of FIP1L1-PDGFRA associated disease, treatment with imatinib is recommended even in the absence of clinical complications at the time the mutation is discovered; i.e. for the rare patients with still asymptomatic hypereosinophilia. There is some debate on the optimal dosing regimen, since doses as low as 100 mg once weekly have been shown to maintain clinical and molecular remission. However, the fact that imatinib treatment interruption in patients who have achieved complete molecular remission is systematically followed by reappearance of the fusion gene suggests that a small, undetected contingent of mutated stem cells persists during treatment, and it is reasonable to fear that imatinib-resistant sub-clones could emerge in patients under suboptimal treatment conditions.[8] Hence, doses below 100 mg daily are not recommended, and some authors even recommend higher dosing if treatment is well tolerated. In pratical terms, the ideal dose of imatinib in a given patient is that required to induce and maintain molecular remission. Although eosinophil levels generally plummet within days in patients with PDGFRA rearrangements, disappearance of the molecular defect generally takes several months.

Besides the FIP1L1-PDGFRA rearrangement, other fusion partners for PDGFRA have been reported in individual cases presenting as HES; all of which have responded dramatically to imatinib. However, molecular investigations on a case-by-case basis such as those reported in these studies are currently only feasible in highly specialized laboratories with a special interest in myeloproliferative disorders. Furthermore, several published open-label trials suggest that a small proportion of patients with persistent unexplained hypereosinophilia may respond to imatinib, in the absence of detectable rearrangements involving PDGFRA.[9] It is, therefore, reasonable to propose a short trial with imatinib in patients with idiopathic HES, especially those with features of myeloproliferative disease. Higher doses of imatinib are generally required to observe a response in the absence of FIP1L1-PDGFRA,[9] so the initial dose should be 400 mg. Moreover, the response may be delayed compared to patients with the mutation, so it may be necessary to combine with other agents (namely corticosteroids) to prevent complications due to uncontrolled eosinophilia at imatinib initiation.

The other major mechanism involved in HES pathogenesis described so far is polyclonal eosinophil expansion in response to IL-5 in the setting of a primitive T-cell disorder. The first case report was that of a young male patient with hypereosinophilia, high serum IgE and IgM levels, and cutaneous, pulmonary, and vascular involvement.[10] The association of high eosinophil and IgE levels led the authors to hypothesize that Th2 cells could be implicated, in an era when the Th1-Th2 paradigm was gaining momentum in human biology. Investigation of T cells in this patient led to the discovery of a phenotypically abnormal subset of CD4 cells lacking membrane expression of the TCR/CD3 complex, and producing IL-4 and IL-5 *in vitro*, both spontaneously and following polyclonal stimulation. This $CD3^{-}CD4^{+}$ T-cell subset was shown to be monoclonal. A follow-up study including 3 additional patients showed that the $CD3^{-}CD4^{+}$ T cells express activation and memory markers, and express neither CD7 nor CD27.[11] Clonality could be demonstrated in all cases, although for one patient with a smaller proportion of aberrant T cells, a polyclonal TCR gene rearrangement pattern was observed by PCR on whole blood, and the $CD3^{-}CD4^{+}$ T cells had to be isolated to observe a clonal band. Another important finding reported in this study was the development of T-cell lymphoma in 2 patients initially presenting with *benign* clonal $CD3^{-}CD4^{+}$ T cells; the patient in the pioneer case report developed splenic anaplastic lymphoma, and one other patient developed peripheral T-cell lymphoma with lymphadenopathy six years after diagnosis of HES.

A number of similar observations have been reported in the literature,[12] and a tentative definition of *lymphocyte-variant* HES (L-HES), or *T-cell mediated HES*, has been proposed, wherein hypereosinophilia is secondary to IL-5 overproduction by an expanded population of T cells which can generally be detected on the basis of an aberrant phenotype. The estimated prevalence of this variant is likely between 17% (Ogbogu *et al.*, submitted for publication) and 26%.[13] It has now become common practice to perform T-cell phenotyping and to investigate TCR gene rearrangements on peripheral blood and, eventually, on bone marrow in patients with HES in order to identify those with L-HES. However, this more thorough approach to HES diagnosis has somewhat obscured our initial understanding about this variant, as illustrated by the study published by Helbig *et al.* in this issue of Haematologica.[14] The authors report a very high incidence of T-cell clonality in peripheral blood of untreated patients with HES (18/42 patients), using a modern, sensitive, and well-accepted technique for investigation of TCR gene rearrangements. Among the

18 patients with clonal TCR gene rearrangement patterns, only one had a previously well-defined phenotypically aberrant T-cell subset ($CD3^-CD4^+$ cells), and for 2 others, T-cell phenotyping showed an unusual distribution of T-cell markers: one patient had clear-cut lymphocytosis involving 98% clonal CD4 T cells, and one had a population of $CD3^+CD8^+CD56^+CD57^+$ T cells representing 32% of all lymphocytes. These results raise several important questions and highlight the gaps in our understanding of the role played by T-cells in pathogenesis of HES. First, what does it mean when phenotyping reveals expanded T-cell subsets other than the most frequently described $CD3^-CD4^+$ population? Second, is demonstration of T-cell clonality alone sufficient to conclude that hypereosinophilia is T-cell mediated in a given patient? And at the end of the day, how should one approach diagnosis of L-HES based on results of these tests?

Past studies have shown that other so-called *phenotypically aberrant* T-cell subsets, which are not normally observed in healthy subjects (or only in small proportions), may be detected in blood from patients with HES. For some of these subsets, including $CD3^+CD4^-CD8^-$[13] and $CD3^+CD4^+CD7^-$[15] cells, their ability to produce IL-5 and/or IL-4/-13 has been demonstrated *in vitro*, indicating their likely involvement in hypereosinophilia. The patient with marked expansion of clonal CD4 T cells bearing an otherwise normal phenotype reported here[14] represents yet another situation, wherein it is very tempting to speculate that this subset is responsible for hypereosinophilia. Although the authors didn't investigate cytokine production by CD4 T cells *in vitro*, the patient's serum IL-4 level was increased, indicating possible overproduction of Th2 cytokines *in vivo*. Similarly, we have recently investigated T cells in a young female patient with marked hypereosinophilia, increased serum IgE levels, an erythematous rash, angioedema, and Raynaud's phenomenon (F. Roufosse, unpublished data, 2008). Phenotyping revealed CD4 T cell lymphocytosis, and membrane staining with a panel of antibodies directed against TCR Vβ family members showed expansion of a Vβ4 subset, with a very slight decrease in staining intensity for CD3, and an increase in staining intensity for CD2. Cultured cells produced IL-13 *in vitro*, but levels of IL-4 and IL-5 were comparable to healthy subjects. Such observations suggest that IL-5 may not be the only cytokine involved in eosinophilic expansion in patients with HES; perhaps the other Th2 cytokines IL-4 and IL-13, or the eosinophilopoietic cytokines IL-3 and GM-CSF, or even other cytokines and growth factors which have not yet been studied in this setting, play a more important role than thought until now.

The pathogenic role of the CD8 T-cell subset reported by Helbig in this issue is more debatable, as there is no evidence here for Th2 cytokine production.[14] Other groups have reported expanded CD8 populations with aberrant surface markers in patients with HES,[13,15] name-

Table 1. Classification of hypereosinophilic syndromes.

	Mechanism of hypereosinophilia	Diagnostic approach	Estimated frequency
HES with well-characterized pathogenesis			
PDGFRA associated HES or CEL	Clonal hypereosinophilia due to acquired autonomous TK activity.	FISH, showing CHIC-2 deletion. PCR amplification of fusion gene. Serum vitamin B12, tryptase	11[1]-14%
	Mechanisms of preferential eosinophil expansion in humans still incompletely understood.	T-cell phenotyping and *TCR* gene rearrangement studies.	17%[1]
Lymphocytic variant HES	Polyclonal hypereosinophilia in response to IL-5 production by activated (clonal) T-cell subsets.	Cytokine production by cultured T cells. Possibly serum TARC level (serum Th2 cytokine levels not shown to be useful).	
Different forms according to phenotype: - CD3-CD4+ - CD3+CD4-CD8- - CD3+CD4+CD7-	Unknown molecular mechanisms leading to acquisition of abnormal phenotype by T cells and deregulated Th2 cytokine production.		
HES with unknown mechanisms of hypereosinophilia			
Suspected "myeloproliferative" forms of HES	Unknown, but eosinophilia assumed to be clonal and mediated by hematopoïetic stem cell mutation involving myeloid lineage	Serum vit B12, organomegaly, altered CBC, immature precursors in blood, increased tryptase, excellent response to imatinib.	unknown
Suspected "T-cell mediated" HES	Unknown, but eosinophilia assumed to be reactive Indirect, incomplete evidence for involvement of T cells	Increased serum TARC, and other features suggesting possible T-cell deregulation (clonal *TCR* gene rearrangement, increased Th2 cytokine production, very high serum IgE, polyclonal hypergammaglobulinemia).	unknown
Idiopathic		Biological and clinical findings not strongly suggestive of underlying myeloproliferative or T-cell mediated disorders.	Majority of patients fulfilling Chusid's criteria

[1]*on the basis of the recent multi-center study, Ogbogu et al., submitted for publication.*

ly 2 $CD3^+CD8^+CD5^{lo}$ subsets with demonstrated clonality, one $CD8^+CD6^{hi}$ and one $CD3^{lo}CD8^{hi}CD6^{lo}$ without clonal TCR rearrangements. In one paper, their presence could be inferred by a low CD4/CD8 ratio (<1).[13] These cells were not shown to produce Th2 cytokines on a cell-by-cell basis, although in one case with $CD3^+CD8^+CD5^{lo}$ cells, cultured PBMC produced detectable IL-5 in the absence of stimulating agents;[13] their pathogenic role in HES, therefore, requires further study. CD8-driven eosinophil expansion in HES is certainly plausible, as non-cytotoxic CD8 T cells with a type 2 cytokine profile have been cloned from peripheral blood of HIV-infected patients, and shown to favor IgE production. Such cases indicate that phenotypes and cytokine profiles of disease-inducing T cells in patients with T-cell driven HES remain poorly defined, and illustrate the emerging heterogeneity within this recently defined HES variant, which likely encompasses several disease forms.

Regarding the issue of whether *isolated* T-cell clonality (i.e. without associated T-cell phenotype abnormalities or demonstration of Th2 cytokine production) is sufficient to consider diagnosis of L-HES, the answer is definitely no, as clonal *TCR* gene rearrangement patterns may be observed in reactive conditions, and even in clear-cut myeloproliferative disease. Indeed, in this issue of the journal, Helbig *et al.* have detected T-cell clonality in 2 patients with the FIP1L1-PDGFRA mutation.[14] Clonal involvement of T cells together with myeloid cells (namely eosinophils) following occurrence of the mutation in a hematopoïetic stem cell is strongly supported by disappearance of the clonal TCR rearrangement pattern in one FIP1L1-PDGFRA positive patient treated with imatinib,[16] and by the occasional combined occurrence of FIP1L1-PDGFRA-associated HES/CEL with lymphoblastic T-cell lymphoma[17] and lymphomatoid papulosis.[18]

That being said, some patients with isolated clonal TCR rearrangements have strikingly elevated serum levels of the chemokine TARC, which is known to be implicated in Th2-mediated diseases. Indeed, we first reported that patients with $CD3^-CD4^+$ T-cell subsets had markedly increased serum TARC levels, and that this was related to production of IL-4 and/or IL-13 by these cells,[19] which may induce TARC production by resident cells and antigen-presenting cells in tissues. We then extended this observation to a larger group of patients with HES,[20] and showed that for several subjects with high serum TARC levels, lymphocyte phenotyping was normal; however, the possibility of an underlying T-cell disorder was suggested by T-cell clonality (shown by PCR for *TCR* gene rearrangements, or by flow cytometry staining for Vβ family members) and/or increased IL-5 production by PBMC *in vitro*. Since these reports, we have observed increased TARC levels in some patients with HES in the complete absence of evidence of T-cell deregulation on the basis of phenotyping, assessment for clonality, and cytokine production by PBMC. The possible involvement of Th2 cells in the induction of hypereosinophilia in such patients remains entirely to be investigated. So, although demonstration of T-cell clonality in the absence of overt phenotype abnormalities is by no means sufficient to conclude that eosinophilia is T-cell driven in a given patient, associated increases in serum TARC and/or Th2 cytokine production by T cells in some cases should be considered as additional evidence for a form of L-HES. It is likely that such situations will be increasingly encountered in the near future, as highly sensitive methods for detection of T-cell clonality have become more readily available.

The high proportion of HES patients with circulating T-cell clones reported in this issue by Helbig is indeed likely representative of T-cell profiles that will be observed in this patient population, using modern approaches such as the BIOMED-2 multiplex PCR protocol, for detection of T-cell clonality. These recommendations have been developed in order to better detect T-cell clonality in blood, marrow, and tissue samples from patients with mature T-cell lymphoproliferative disorders (e.g. peripheral T-cell lymphoma, angioimmunoblastic T-cell lymphoma), whose diagnosis is extremely challenging, and frequently delayed despite a high level of clinical suspicion in many cases.[21] Simultaneous testing for clonal TCR beta, gamma, and delta rearrangements using a large number of primers, and denaturation/renaturation of PCR products for heteroduplex analysis, have increased the diagnostic sensitivity for these disorders, while retaining the desired specificity with regard to healthy subjects (i.e. polyclonal rearrangements). However, detection of T-cell clonality using the BIOMED-2 protocol in the setting of chronic and acute inflammatory disorders does not necessarily indicate a primary pathogenic role for T cells; and the specificity for a true T-cell lymphoproliferative disorder among patients with hypereosinophilia remains to be evaluated prospectively. Until recently, interest for interactions between T cells and eosinophils has focused mainly on the effects of T cells on eosinophil biology, in the setting of reactive hypereosinophilia, but the effects that eosinophils exert on T cells are only beginning to be investigated. It has clearly been shown that eosinophils can act as antigen presenting cells,[22] and the recent development of an eosinophil-deficient transgenic strain of mice (PHIL) has led to the unexpected and intriguing observation that eosinophils are critical orchestrators of allergic airway inflammation in mice, required for the localized recruitment of allergen-specific effector T cells.[23] Thus, the possible role played by eosinophils in clonal T-cell expansion associated with chronic hypereosinophilia is a plausible hypothesis which deserves investigation.

Given this, the conclusion can be drawn that formal diagnosis of L-HES *currently* requires careful T-cell phenotyping and PCR analysis of TCR gene rearrangements, ideally in conjunction with assessment of cytokine production by cultured PBMC or T cells. An exception to this last requirement is made for patients with T-cell subsets bearing previously well-characterized phenotypic abnormalities, and shown by others to produce Th2 cytokines, such as $CD3^-CD4^+$, $CD3^+CD4^-CD8^-$, and perhaps expanded $CD3/CD4^+CD7^-$ subsets. Detection of high serum TARC levels, considered a hallmark of Th2 cytokine production *in vivo*, may

also be indicative of T-cell driven hypereosinophilia, and should prompt careful analysis of the above-mentioned tests, as well as thorough investigation for T-cell lymphoma. In the absence of abnormal findings, exploratory investigation of cytokines and growth factors which could be implicated in eosinophil expansion should be conducted in specialized centers. As knowledge on T-cell mediated HES evolves, so will the recommendations for interpretation of these tests; and new biomarkers with higher sensitivity and specificity may be identified.

The current struggle to delineate L-HES defining features is intimately linked with the lack of knowledge in terms of primary molecular mechanisms involved in this group of eosinophil-associated T-cell lymphoproliferative disorders. Investigations are further complicated by the increasing heterogeneity within this group, and it is unclear whether observations made on a specific subset (e.g. $CD3^-CD4^+$ T cells) will be relevant for the others. It has been reported that for $CD3^-CD4^+$ T cells, absence of the CD3/TCR membrane complex is related to suppressed transcription of the CD3γ chain gene, and that this may be due to increased binding of NFATc2 to the CD3γ promoter;[24] however, mechanisms leading to NFATc2 overexpression remain elusive. Another study has shown that $CD3^-CD4^+$ T cells from 2 patients with L-HES contained partial 6q deletions, and that in one case, progression towards T-cell lymphoma was associated with dominant emergence of a specific 6q-deleted (6q13q22) subclone.[25] Micro-array and RT-PCR studies comparing $CD3^-CD4^+$ cells to normal $CD3^+CD4^+$ cells, and focusing on genes located on this 6q segment, showed decreased expression of a series of genes in the former, the functional relevance of which remains to be studied. It has been speculated that inactivation of tumor suppressor gene(s) present in the 6q region may be an early step in the progression towards lymphoma. Another proposed mechanism leading to expansion of abnormal T-cell subsets associated with HES is deficient Fas-mediated apoptosis, either through absence of membrane CD95 (Fas-R),[13] or through transcription of a Fas-R splice variant encoding a shorter soluble protein able to interfere with engagement of normal membrane-expressed Fas-R, as demonstrated in one patient with clonal $CD3^+CD4^-CD8^-$ cells.[26] More recently, a study comparing the gene expression profile of CD3-$CD4^+$ T cells to $CD4^+$ T cells from healthy subjects using high-density micro-array chips has pointed towards deregulated expression of molecules involved in important homeostatic pathways.[27] The functional relevance of these results is currently under investigation.

Thus, in contrast to FIP1L1-PDGFRA associated disease, development of targeted therapy for T-cell driven HES remains hindered by our incomplete understanding of primary molecular mechanisms, and specific pathways involved in survival, growth, and persistent activation of the abnormal T-cell subsets. In the meantime, corticosteroids (CS) remain first-line therapy for patients with L-HES, and absolute numbers of CD3-$CD4^+$ T cells have been shown to decrease in some patients treated with CS alone.[12] Second-line and/or CS-sparing therapeutic options for patients with L-HES include interferon-alpha (IFN-α), which has mostly been reported successful for treating HES patients with features of myeloproliferative disease, and possibly alemtuzumab.[12] IFN-α may induce partial regression of pathogenic $CD3^-CD4^+$ T cells,[10,13] and we have observed complete disappearance of $CD3^-CD4^+$ cells in one patient treated with combined CS and IFN-α (F. Roufosse, 2004, unpublished observation). Association with a pro-apoptotic agent for abnormal T cells, like CS, is recommended, due to the survival-promoting effect of IFN-α on $CD3^-CD4^+$ T cells observed *in vitro*. Alemtuzumab targets the CD52 antigen, which is expressed both on T cells and eosinophils, and is, therefore, appealing for L-HES treatment, but the risk/benefit ratio must be examined closely on a case-by-case basis in light of the marked immunosuppression it entails. An interesting approach in the setting of L-HES may be tailored dosing of alemtuzumab, based on the absolute numbers of aberrant T cells in peripheral blood, as recommended by one group for patients with Sézary syndrome. Finally, mepolizumab, a monoclonal anti-IL-5 antibody, has recently been shown to enable CS-tapering while maintaining disease control and eosinophil depletion in a high proportion of patients with HES, with little if any side effects compared to placebo.[28] Efficacy in patients with L-HES remains to be evaluated separately, and although one would anticipate beneficial effects on IL-5-driven hypereosinophilia, anti-IL-5 treatment is unlikely to affect pathogenic T cells.

Once full-blown peripheral T-cell lymphoma has developed in patients initially diagnosed with L-HES, eradication of malignant T cells is not easily achieved using classical chemotherapeutic regimens. We and others have reported that intensification of chemotherapy followed by transplantation of allogeneic stem cells successfully and durably eradicated the malignant $CD3^-CD4^+$ T-cell clone in 2 cases.[12,29]

In conclusion, improved understanding of HES pathogenesis in patient subgroups has modified management of this chronic and often debilitating disorder. Discovery of a disease-inducing mutation involving an imatinib-sensitive tyrosine kinase has spectacularly reversed natural disease course in affected patients. In contrast, development of novel targeted therapy for T-cell mediated HES (L-HES) is precluded by the emerging complexity and heterogeneity within this variant, and the current lack of insight regarding underlying molecular events leading to T-cell deregulation. Therapeutic recommendations have been somewhat modified nonetheless, favoring agents which target T cells as well as eosinophils. Future directions for research which are essential for improved treatment and outcome of this HES variant include evaluation, standardization, and validation of modern diagnostic methods, identification of biomarkers for diagnosis and malignant progression, and investigation of primary molecular mechanisms of disease.

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