Genetic and phenotypic characterization of the lymphocytic variant of the hypereosinophilic syndrome: a model of T lymphomagenesis

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The lymphocytic variant of the hypereosinophilic syndrome (L-HES) is a rare disease characterized by symptoms linked with the persistence and proliferation of a $CD4^+$ T-cell clone producing cytokines that induce polyclonal hypereosinophilia. The work presented in this thesis specifically addresses the question of the mechanisms that are deregulated in the $CD3^-CD4^+$ T cells allowing them to expand in L-HES patients and put them at significant risk for the development of a T-cell lymphoma. The first aim of this study was to characterize the phenotypic and immunological traits of the $CD3^-CD4^+$ T cell clone found in the majority of L-HES patients. The second aim of this work was to identify the genetic alterations associated with the sustained survival and expansion of the L-HES T-cell clone. Our ultimate goal was to identify potential tumor suppressor genes and decipher the role they play in the normal apoptotic and proliferative mechanisms responsible for controlling $CD4^+$ T cell growth.
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Introduction

Hypereosinophilic syndrome (HES) is an orphan disease that includes a heterogeneous group of clinical conditions characterized by morbid and persistent hypereosinophilia as a common denominator.[1] After excluding all sources of secondary hypereosinophilia, six distinct clinical entities of HES have been described.[2] Pathophysiologically, two major types, the myeloid (M-HES) and the lymphocytic (L-HES) variants are easily distinguishable.[3] The M-HES variant can be molecularly defined by the platelet-derived growth factor receptor (*PDGFRA/B*) fusion gene detected in a neoplastic myeloid-lymphoid precursor stem cell. These aberrant precursor cells express an activated tyrosine kinase that is sensitive to imatinib.[4] In contrast, the lymphocytic variant (L-HES) is characterized by reactive hypereosinophilia secondary to the clonal expansion of an aberrant $CD4^+$ T-cell population, which secretes high levels of eosinophilopoietic cytokines (IL5, IL-13).[5] Three types of L-HES clones have been defined including $CD3^-CD4^+CD8^-$, $CD3^+CD4^-CD8^-$ and $CD3^+CD4^+CD7^-$.[6-8] The most prevalent is the $CD3^-CD4^+CD8^-$ subset representing 60% of the L-HES T cell clones.[9] L-HES patients benefit from

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symptomatic treatment such as corticosteroids and mepolizumab that are targeted to eosinophil reduction and their consequent tissue damage.[10] Although the short-term clinical evolution of L-HES appears to be benign, several patients in our study developed T-cell lymphomas with variable latency.[9] This predisposition to T lymphomagenesis in L-HES patients therefore presents an invaluable model for studying the underlying genetic events leading to $CD4^+$ T-cell transformation and underscores the need to identify patients at risk for effective targeted treatments.

Material and Methods

The first objective of this study was to fully characterize the phenotype and the immunological properties of the $CD4^+$ T cell-clones from L-HES patients diagnosed in collaboration with Prof. M. Goldman and Dr. F. Roufosse (Department of Internal Medicine, Hôpital Erasme, Brussels and Institute for Medical Immunology (IMI), Gosselies, ULB). This objective was achieved by producing a complete profile of the $CD3^-CD4^+$ T-cells, including their immunophenotype, cytokine and chemokine secretion pattern, infectious status and T cell receptor rearrangement. The second objective of this work was to identify the genetic aberrations linked with the sustained survival and expansion of the $CD3^-CD4^+$ T-cell clone. This goal was accomplished at the chromosomal, molecular and cellular levels by assessing the karyotype, the evolving molecular profiles as well as designing functional experiments to characterize the mechanisms that underlie the persistent expansion of the abnormal $CD4^+$ T-cell clone.

Results

Phenotypic characterization of the aberrant Th2 clones from L-HES patients provided a detailed immunophenotype of the $CD3^-CD4^+$ T-cells, both at diagnosis and during follow-up as their disease evolved. Substantial similarities and remarkable stability of the surface immunophenotype was detected for the $CD3^-CD4^+$T-cells from different L-HES patients. Furthermore no underlying viral infection was detected in the abnormal cells in spite of the frequent loss of TCR/CD3 expression in a variety of viral based diseases (HIV, HTLV-I, etc.). The reproducible immunophenotype for the aberrant T cell clone in majority of our patients was $CD2^+CD3^-CD4^+CD5^+CD7^-CD25^-CD28^+CD45RO^+CD62L^+CD69^-$.[11]

Cytogenetic analyses of $CD3^-CD4^+$ T cells from two L-HES patients (P1 and P2) performed in parallel revealed a common 6q13-q22.1 chromosomal deletion.[9] Retrospectively, studying the progression timeline for these cytogenetic aberrations during their clinical evolution revealed a persistent 6q- interstitial deletion present in both patients during the chronic phase of L-HES. Furthermore, the emergence of a predominant 6q- subclone as P1's disease progressed to T-lymphoma suggested a critical role for 6q-located gene(s) in malignant transformation.[12]

Our next goal was to detect and identify the critical molecular changes in $CD3^-CD4^+$ T-cells in association with chronic disease and the evolution to T lymphoma. We used whole genome gene expression arrays to compare $CD3^-CD4^+$ T-cell clones from three patients relative to $CD3^+CD4^+$ T-cells from healthy controls.[13] These data, confirmed by quantitative RT-PCR, established a molecular profile for the $CD3^-CD4^+$ T-cells that was characterized by their Th2 memory phenotype and an inflammatory profile. In addition, these data revealed deregulated expression of genes involved in signaling pathways, including TGFb and apoptosis/survival mechanisms, suggesting that important changes had occur in the immune and homeostatic regulation of these cells.[13]

The recurrent detection of 6q deletions in a variety of solid tumors and lymphoid diseases strongly suggests the presence of one or more tumor suppressor genes residing on the long arm of chromosome 6.[14-16] Our final objective was to identify potential suppressor gene(s) in the 6q13-22.1 region. Toward this goal, the genes present in the deleted region were examined for reproducible changes in the transcription profiles of $CD3^-CD4^+$ T-cells obtained from patients during chronic disease and as one patient progressed to T-lymphoma. The microarray and qRT-PCR results revealed the continuous repression of only one 6q located gene, *BACH2*, as P1's disease progressed. In combination with data from the literature our data identified *BACH2* as the most relevant candidate tumor suppressor gene located in the 6q deletion.

Figure 1. Model for L-HES progression to T lymphoma.

Th2 polarization		L-HES		persistent L-HES		T Lymphoma
	step 1		step 2		step 3	
$CD3^+CD4^+$ T cell	→	TCR clonality + CD loss	→	BACH2↓./ 6q- (Fas-L resistance)	→	further mutations (RUNX2/PRDM1…)

We performed functional analyses using shRNAs to silence *BACH2* expression and these data confirmed its suppressive properties by demonstrating it has the ability to modulate apoptosis in $CD4^+$ Jurkat T-cells subjected to genotoxic stress. We further found that this suppression of apoptosis (both in Jurkat and $CD3^-CD4^+$ cells from patients) was mediated via transcriptional regulation of the *FAS-L* gene. These data provide new insight into $CD4^+$ T lymphomagenesis. FAS-L is a pivotal mediator of activation-induced cell death (AICD), suggesting that the BACH2 deletion may confer greater survival on the $CD3^-CD4^+$ T cells, with their chronic proliferation provoking the associated hypereosinophilia and their abnormal persistence providing a predisposition to T lymphoma transformation *in vivo*.[17]

Conclusions

The identification of the 6q-located *BACH2* as a haploinsufficient tumor suppressor gene in $CD4^+$ T cells may provide the foundation for a new model of T lymphomagenesis. Our data provide evidence that the suppressive effect of BACH2 is mediated via *FAS-L*, a major player in the activation-induced cell death (AICD) pathway, and suggest that BACH2 plays a critical role in $CD4^+$ T-cell homeostasis. These data constitute the first experimental evidence that BACH2 exerts a regulatory effect on the FAS-L extrinsic apoptotic pathway in $CD4^+$ effector memory T-cells.

In a previous study, we found that miR-125a levels were significantly reduced in our entire L-HES patient cohort relative to controls with a further reduction detected as P1 evolved to T lymphoma.[13] miR-125a has recently been shown to regulate hematopoietic stem cell regeneration of hematopoiesis, in part by reducing apoptosis[18]. The anti-apoptotic effect of miR-125a functions by downregulating the pro-apoptotic protein Bak1. Although we did not detect changes in *BAK1* expression in the abnormal $CD3^-CD4^+$ T cells from our L-HES patients, this does not exclude the possibility that miR-125a also acts either directly or indirectly on other genes involved in regulating apoptosis, potentially including BACH2. These observations suggest that the deregulation of key regulators of T cell expansion and controlled post-activation reduction have been re-wired in the $CD3^-CD4^+$ T cells favoring their abnormal persistence and the eventual outgrowth of a malignant cell clone.

Taken together, our data show how the *BACH2*-deficient L-HES $CD4^+$ T-cells that are unable to undergo FAS-L mediated apoptosis persist and expand, producing Th2 cytokines upon stimulation and thereby provoking the secondary hypereosinophilia. These constant rounds of expansion favor the accumulation of additional abnormalities that eventually lead to full-blown T lymphoma in some patients. This work identifies potential therapeutic and diagnostic targets that can now be investigated for L-HES as well as other autoimmune, lymphoproliferative and infectious diseases where the $CD4^+$T-cells homeostasis is altered.

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