Unexplained Hypereosinophilia and the Need for Cytogenetic and Molecular Genetic Analyses

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Background: Idiopathic hypereosinophilic syndrome (HES) is a diagnosis made after the exclusion of other causes of eosinophilia. However, differentiation of idiopathic HES from eosinophilic leukemia is sometimes difficult. In some cases, these diagnoses can be differentiated by cytogenetic or molecular findings, as illustrated in the patients described herein.

Observations: We describe 3 patients with HES and associated pruritus; 1 patient also had recurrent lesions of eosinophilic cellulitis. All 3 patients were initially diagnosed as having idiopathic HES, but after evaluation and demonstration of molecular abnormalities, they were classified as having eosinophilic leukemia.

Conclusions: Patients with a diagnosis of idiopathic HES should be evaluated for cytogenetic or molecular genetic abnormalities. These abnormalities can establish a diagnosis of chronic eosinophilic leukemia and may provide clues for emerging therapies.

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DIOPATHIC HYPEREOSINOPHILIC syndrome (HES) is characterized by unexplained hypereosinophilia (eosinophil counts >1500/µL persisting for ≥6 months) that may lead to organ damage. At times, idiopathic HES may be difficult to differentiate from chronic eosinophilic leukemia (EL). Features that have been said to favor EL include hepatomegaly, splenomegaly, anemia, and thrombocytopenia. However, the diagnosis of EL can be made if blast crisis or a soft tissue tumor of myeloblasts (granulocytic sarcoma) develops, if a clonal eosinophilic proliferation is found, or if characteristic molecular or cytogenetic abnormalities are present.

All 3 patients were initially diagnosed as having idiopathic HES. Further hematologic workup showed a chromosomal translocation in 1 patient. A characteristic chromosomal deletion seen in many patients with HES was found in the other 2 patients. Both abnormalities can result in constitutive activation of tyrosine kinase pathways and are consistent with the uncontrolled growth seen in chronic EL. The constitutive activation of tyrosine kinase pathways suggests that inhibitors of these pathways could have a role in therapy. The underlying molecular and chromosomal abnormalities may also have implications for the initial response and for the duration of response.

REPORT OF CASES

All 3 patients had at least 2 documented absolute peripheral blood eosinophil counts greater than 1500/µL for greater than 6 months (Table). Initially, all 3 patients had negative results of evaluations for a known cause of eosinophilia, including an evaluation for parasitic infections, and clinical or laboratory findings consistent with eosinophilic disorders, including Churg-Strauss vasculitis, chronic eosinophilic pneumonia, eosinophilic gastroenteritis, and episodic angioedema and eosinophilia. All 3 patients had histologic evidence of tissue infiltration by eosinophils or objective evidence of tissue damage in an organ system associated with eosinophilia. One patient had pruritus with recurrent erythematous nodules and plaques that showed features consistent with eosinophilic cellulitis (Table and Figure). This patient also had gastrointestinal complaints and splenomegaly on physical examination, which were confirmed by computed tomography. The 2 other patients had pruritus with transient erythematous eruptions. Both these patients had obstructive pulmonary disease on pulmonary function testing, with
Patients With Hypereosinophilic Syndrome With Cytogenetic Changes Consistent With Chronic Eosinophilic Leukemia Responding to Imatinib Therapy

<table>
<thead>
<tr>
<th>Patient Age, y/ Sex/Race</th>
<th>Clinical Findings</th>
<th>Previous Therapy</th>
<th>Imatinib Mesylate, mg/d</th>
<th>Cytogenetic Findings</th>
<th>Baseline Eosinophil Counts, /µL (%</th>
<th>Duration of Response, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.56/M/W</td>
<td>Eosinophilic cellulitis, pruritus, GI symptoms, splenomegaly</td>
<td>Prednisone, hydroxyurea,* interferon alfa</td>
<td>600</td>
<td>t(5;12)† (q33;p13)</td>
<td>42.3 (40)</td>
<td>4†</td>
</tr>
<tr>
<td>2.46/M/W</td>
<td>Pruritus, transient rash, pulmonary infiltrates, deep vein thrombosis</td>
<td>Prednisone</td>
<td>400</td>
<td>FIP1L1-PDGFRα</td>
<td>29.2 (32)</td>
<td>&gt;8</td>
</tr>
<tr>
<td>3.52/M/W</td>
<td>Pruritus, transient rash, pulmonary infiltrates, peripheral neuropathy</td>
<td>Prednisone</td>
<td>400</td>
<td>FIP1L1-PDGFRα</td>
<td>15.3 (23)</td>
<td>&gt;7</td>
</tr>
</tbody>
</table>

Abbreviations: GI, gastrointestinal; W, white.
*Controlled disease for approximately 18 months.
†Cytogenetic findings found at the time of original diagnosis, 3 years earlier.
‡Relapse with blast crisis. The patient is now taking flavopiridol and depsipeptide.

COMMENT

Hypereosinophilic syndrome is a rare hematologic disorder characterized by sustained overproduction of eosinophils in the bone marrow, eosinophilia, tissue infiltration, and organ damage. Hypereosinophilia (>1500/µL for >6 months) occurs in the absence of other causes of eosinophilia, including parasitic infections, immune dysregulation, and allergies. Other symptoms of organ involvement affect the heart, lungs, gastrointestinal system, central and peripheral nervous systems, and skin. Total leukocyte counts are usually less than 25000/µL, with 30% to 70% eosinophilia, and bone marrow eosinophils are increased 30% to 60%; rarely, there are myeloblasts. Although it can be difficult to assess the clonality of HES, rare cases show karyotypic abnormalities. In female patients, clonality by investigation of X-linked polymorphisms of the phosphoglycerate kinase genes and the human androgen receptor gene (HUMARA) can be performed. A skewed distribution of expression of X chromosome genes provides presumptive evidence of clonality and neoplasia; however, 90% of patients with idiopathic HES are males, and, thus, X-linked polymorphisms are not applicable in most patients. In rare cases of HES, a clone of T cells or, more rarely, a polyclonal T proliferation secretes cytokines that induce hypereosinophilia. The interleukin 5 concentration is usually elevated, but increased levels of interleukin 4 and granulocyte-macrophage colony-stimulating factor have also been reported. Clonal and interstitial infiltrates on chest computed tomographic scans; in addition, 1 patient had an episode of deep vein thrombosis and the other had progressive peripheral neuropathy (Table).

The first patient had been diagnosed more than 3 years earlier and was initially treated with prednisone, but periodic flares became persistent. After approximately 1 year, the patient underwent cytogenetic studies that showed chromosomal translocation t(5;12)(q33;p13). The patient was then given a presumptive diagnosis of chronic EL and was treated with hydroxyurea for approximately 14 months. Although the patient initially did well, his response was short-lived, and he again developed symptoms, including recurrent lesions of eosinophilic cellulitis and hypereosinophilia; he was administered interferon alfa without response. The patient was then given imatinib mesylate (Gleevec; Novartis Pharmaceuticals Corp, East Hanover, NJ), 600 mg/d, with initial rapid resolution of symptoms and eosinophilia. After 4 months of imatinib therapy, the patient experienced blast crisis and was administered flavopiridol and depsipeptide.

In patients 2 and 3, cytogenetic analysis of unstimulated bone marrow cells showed no evidence of a clonal hematopoietic process. In addition, patients 2 and 3, results of polymerase chain reaction (PCR)–based T-cell gamma receptor and immunoglobulin heavy chain gene rearrangement analysis of peripheral blood mononuclear cells or bone marrow aspirates and flow cytometric analysis of T-cell surface markers were negative for clonality and an aberrant surface profile. RNA was then isolated from peripheral blood mononuclear cells of both patients using TRizol Reagents (Invitrogen Corp, Carlsbad, Calif) and analyzed by nested PCR using primers FIP1L1-F1 (5’-actgctcgctgttctgat) and platelet-derived growth factor receptor (PDGFR) α-R1 (5’-ttggagcttttctgagga) during the first PCR and primers FIP1L1-F2 (5’-aaagatgccggcggactc) and PDGFRα-R2 (5’-ggagccggatcatcag) for the second PCR. Control reverse transcriptase PCR for GAPDH was performed using the primers GAPDH (glyceraldehyde-3-phosphate dehydrogenase)-F (5’-tggaataccctaccaacctcct) and GAPDH-R (5’-gtcttcggcctgctgcatc). Products of PCR were cloned in the pGEM-T Easy Vector System (Promega Corp, Madison, Wis), and cloned products were sequenced on an ABI system (PerkinElmer Inc, Boston, Mass). Both patients showed fusion of the FIP1L1 gene to the PDGFRα.

Patients 2 and 3 were subsequently given imatinib mesylate, 400 mg daily, and they both had rapid clearing of symptoms with normalization of eosinophil counts; they have remained clear for more than 8 months, with no apparent adverse effects from the medication.

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polyclonal T-cell proliferations show an abnormal phenotype CD3−CD4+ or CD3−CD4+CD8−, and both seem to depend on exogenous signals for proliferation and cytokine production.12

Eosinophilic proliferation is common in a variety of defined subtypes of chronic myeloid leukemia, including chronic EL or chronic myelomonocytic leukemia with eosinophilia associated with translocation t(5;12) (q33;p13) and chronic EL with evolution to acute myeloid leukemia and T-lineage lymphoblastic lymphoma associated with t(8;13)(p11;q12) or other cytogenetic aberrations with an 8p1-12 breakpoint.1,13,17 The initial oncogenic event in the development of EL and other chronic myeloid leukemias mainly involves genes for membrane receptors or their signaling pathways, including PDGFRα and PDGFRβ and fibroblast growth factor receptor 1, as well as Janus kinase 2.1 Activation of these pathways results in constitutive activation of tyrosine kinases.1 In contrast, in most de novo acute myeloid leukemias, transcription factor genes are affected.1

The first patient had a previously identified translocation in the myeloid cells, t(5;12)(q33;p13). The likely mechanism of leukemogenesis with this translocation is the fusion of the tel gene and the PDGFRβ, resulting in constitutive activation of the tyrosine kinase domain of PDGFRβ.27 Leukemogenesis is probably mediated through c-Myc because a threshold concentration of active Myc protein is necessary to permit transformation of cells by the fusion gene.18 Rarely, different translocations involving the tel gene, t(9;15)(p24;q15;p13) with Janus kinase 2 and t(5;12)(q31;p13), have also been described. The latter translocation may correspond to activating mutations for the production of interleukin 5 at the 5q31 locus.1 Other cytogenetic abnormalities found in association with chronic EL include fusion of zinc finger 198–fibroblast growth factor receptor 1, trisomy 15, t(8;9) (p22;p23), t(3;9;5)(q25;q34;q33), and t(8;9)(p11;q32-34) and t(6;8)(q27;p12).1,18,19 The latter 2 translocations have a poor prognosis, with a high incidence of acute transformation, and the best therapeutic option for young patients with these translocations is probably stem cell transplantation early after diagnosis.1 There is overlap between eosinophilic myelodysplastic syndrome and chronic EL. However, eosinophilia is uncommon in myelodysplastic syndrome, and the greatest prevalence is in cases of therapy-related myelodysplastic syndrome showing a t(1;7).19 Patients 2 and 3 had a molecular defect similar to that found in greater than 50% of patients originally diagnosed as having idiopathic HES.20 They showed that fusion of the FIP1L1 gene to the PDGFRα with a deletion on 4q12 results in constitutive activation of PDGFRα tyrosine kinase.

Treatment of HES has had as its goal limitation of organ damage by controlling eosinophilia. In idiopathic HES, prednisone, interferon alfa, psoralen–UV-A, hydroxyurea, cyclosporine, and, less commonly, other cytotoxic therapies have been used.21,22 The clinical manifestations and responses to therapy are variable in patients with HES, which may reflect the underlying variable etiology of the disorder. Now that it is realized that some of these patients do in fact have clonal proliferations of myeloid cells that represent chronic EL and not reactive myeloid proliferations, the failure of therapy and the progression in some patients are better understood.

There are no agreed-on guidelines for the treatment of chronic EL.21-23 Although complete remissions have occurred with interferon alfa therapy, these remissions are often short-lived.23 Imatinib, a 2-phenylamino pyrimidine-based tyrosine kinase inhibitor that is approved for the treatment of BCR-ABL–positive chronic myeloid leukemia and acute lymphoblastic leukemia, has recently been used successfully in patients with HES.20,24-26 In addition to the ABL tyrosine kinase, imatinib also inhibits the type III transmembrane receptor KIT and PDGFRs.25-27 Clinical response to imatinib therapy in patients diagnosed as having idiopathic HES led to the discovery that more than 50% of these patients had fusion of the PDGFRα and FIP1L1 genes generated by an interstitial deletion on 4q12. This fusion product results in constitutively activated PDGFR tyrosine kinase activity, which is potentially inhibited by imatinib.25 Several investigative groups27-29 have now confirmed that some cases of HES do respond to imatinib treatment.

Chronic myeloid leukemia, the first disease indication for imatinib therapy, is a myeloproliferative disease that overlaps with EL. Chronic myeloid leukemia is a tri-
phasic disease beginning with a relatively innocuous chronic phase, in which 50% of patients can be asymptomatic for an average of 4 to 5 years, with progression to an accelerated phase lasting 6 to 18 months, and terminating in fatal blast crisis with a duration of approximately 6 months. With progression to blast crisis, the malignant clones acquire additional genetic mutations, including trisomy 8, isochromosome 1 (17q), and trisomy 19. Thus, the therapeutic window for cure is early in disease.

Imatinib binds to tyrosine kinases BCR-ABL, KIT, PDGFRα, and PDGFRβ, blocking the binding of adenosine triphosphate. This prevents the phosphorylation of substrate on the kinases. Imatinib has a low toxicity profile compared with many other cytotoxic agents. The most common adverse reactions are diarrhea, reflux, taste disturbances, peripheral edema, nausea, and skin eruptions in up to 30% of patients. The skin eruptions are mainly maculopapular exanthes; however, Stevens-Johnson syndrome and acute pustular exanthema have been reported. There are also reports of depigmentation, including graying of hair with imatinib therapy. The depigmentation is speculated to be secondary to the inhibition of KIT, which is expressed on skin basal cells, melanocytes, and mast cells. However, there are also reports of hyperpigmentation and darkening of hair color in some patients. In patients taking high doses, 800 to 1000 mg/d, thrombocytopenia and neutropenia have occurred.

Imatinib is metabolized in the liver primarily by cytochrome (CYP)3A4/5. Thus, inducer and inhibitors of this enzyme should be used with caution. Imatinib is a weak inhibitor of CYP2D6 and CYP2C9, and drugs metabolized by these enzymes should be monitored, particularly warfarin, which has been associated with central nervous system hemorrhage with imatinib.

Resistance to imatinib can occur through gene amplifications resulting in higher protein levels and gene mutations in the ATP-binding pocket resulting in the inability of imatinib to competitively bind out ATP. In addition, increased expression of the multidrug resistance (MDR) 1 gene correlates with resistance to imatinib. MDR-1 encodes P-glycoprotein, a member of the ATP-binding cassette protein family that functions to remove toxic molecules from cells. Inherited polymorphisms in the MDR-1 gene may explain some of the variable sensitivity to imatinib in different patients.

The first patient in this study developed resistance to imatinib within 4 months. Perhaps his primary cytogenetic changes or his previous therapies, including hydroxyurea and interferon alfa, predisposed him to more rapid development of resistance with the development of blast crisis.

Genetic and biochemical data support that Ras activation has a central role in the oncogenic mechanisms of the tyrosine kinases that are inhibited by imatinib. The combination of H-ras inhibitors, that is, farnesyl transferase inhibitors with imatinib mesylate, seems to increase the initial response and the duration of response to imatinib. Combinations of imatinib with interferon alfa, vincristine sulfate, daunorubicin hydrochloride, or cytarabine arabinoside, or more novel compounds such as the Janus kinase 2 inhibitor AG490, are also being evaluated, and some of these combinations seem to show some synergy. Treatment with histone deacetylase inhibitors such as depsipeptide and cell cycle modulators such as flavopiridol, as were used in the first patient who entered blast crisis, are also being evaluated in combination with imatinib to increase the its efficacy and delay the development of resistance.

In conclusion, cytogenetic and molecular genetic analyses are probably indicated in all patients who meet the criteria for idiopathic HES, especially since they may define defects that result in activation of molecular pathways for which we now have inhibitors.

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