Cytogenetic and molecular cytogenetic characterization of 6 new cases of idiopathic hypereosinophilic syndrome

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ABSTRACT

Eosinophilia is a hematologic manifestation of many disease states, particularly parasitic infestations, collagen vascular diseases, allergies, and solid tumors such as breast and ovarian cancer and Hodgkin’s disease, or even acute leukemias and myeloproliferative disorders. In a few cases a combination of unexplained prolonged eosinophilia with evidence of organ involvement may occur; this condition is referred to as idiopathic hypereosinophilic syndrome (HES). Three defining features for HES were proposed by Chusid in 1975: 1) sustained blood eosinophilia greater than 1,500 cells/µL lasting for more than 6 months; 2) absence of any known cause of eosinophilia; 3) signs and symptoms of organ involvement. HES may be a reactive condition or a chronic myeloproliferative disorder, in which latter case it is usually referred to as chronic eosinophilic leukemia (CEL). The distinction on clinical grounds between these two entities is not clear, and some authors have suggested that the presence of blasts (>10%) in the bone marrow (BM), the presence of immature eosinophils in different tissues, an aggressive clinical course and the presence of clonal cytogenetic anomalies may be regarded as indicative of a clonal myeloproliferative disorder.

To define the clinicobiological profile of this condition better, morphologic, cytogenetic, and hematologic features in 6 patients fulfilling the above criteria for the diagnosis of HES were analyzed in this study. Interphase FISH studies were also performed, in order to rule out the occurrence of submicroscopic chromosomal lesions involving some regions known to be frequently affected in myeloproliferative disorders. Our data confirm that HES is a clinically and biologically heterogeneous condition and suggest that more cases need to be studied to identify clinically significant chromosome changes in this rare condition. Some patients may benefit from treatment with interferon.

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Key words: HES, FISH, cytogenetics

Design and Methods

Patients

Patients included in the present report were seen at our Institution over a 6-year period. Diagnostic work up included: a) laboratory profile; b) chest X-ray radiography; c) abdominal ultrasonography; d) echocardiogram; e) electrocardiogram; f) bone marrow aspiration and bone marrow biopsy; g) cytogenetic analysis. The presence of parasitic infections, of collagen vascular diseases, and of allergies was excluded in all patients. A summary of the principal clinical parameters is shown in Table 1.

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Original Paper

Stem Cell Disorders
cough. His leukocyte count was 40.1 × 10^9/L in 1994 with a few week's history of fatigue, fever and weight loss, hepatosplenomegaly, hyperleukocytosis (62 × 10^9/L), and eosinophilia (PB count 20%, BM 58% in peripheral blood (PB) and 30% in bone marrow (BM); no dysplastic features were present at morphologic evaluation. A chest X-ray film showed a pulmonary infiltrate. No evidence of involvement of other organs was recorded. The patient was treated with steroids, which proved ineffective at reducing the WBC count. In October 1994 therapy with hydroxyurea was started, with gradual reduction of other organs was recorded. The patient was treated with steroids, which proved ineffective at reducing the WBC count. In October 1994 therapy with hydroxyurea was started, with gradual reduction of eosinophils and resolution of lung involvement.

Case #2 (MO). A 70-year old man presented in March 1995 with dyspnea, fever, leukocytosis (16.7 × 10^9/L), and eosinophilia (PB count 14%, BM 20%). Cardiac, abdominal, skin and neurologic evaluations were unremarkable, but a chest X-ray film showed a pulmonary infiltrate. Methylprednisolone was administered for 3 months, obtaining rapid resolution of the lung involvement and partial control of the PB eosinophilia. The patient is still receiving intermittent steroid therapy and is alive and well with an absolute eosinophil count ranging between 1,200 and 2,000/μL.

Case #3 (BA). A 29-year old man was admitted for the first time in July 1993 because of hyperleukocytosis (199 × 10^9/L), thrombocytopenia (42 × 10^9/L), and eosinophilia (64% in PB and 30% in BM). No blasts were seen on either PB or BM morphologic evaluation. Clinical examination showed splenomegaly (8 cm below the costal margin), while the echocardiogram detected a mild mitral regurgitation. Methylprednisolone therapy was ineffective, obtaining only a transitory reduction of leukocytosis. Therapy with steroids and hydroxyurea was promptly adopted, without satisfactory control of the WBC count. The patient died 13 months after diagnosis.

Case #4 (RA). A 73-year old man presented in September 1994 because of the onset of an erythematous rash and angioneurotic edema. Blood investigation showed 1,800/mm^3 eosinophils. Physical examination documented mild liver and spleen enlargement; no evidence of allergic disease was found. Because of the persistence of eosinophilia and dermatitis, intermittent steroid therapy was started obtaining a 3-year partial remission. In March 1998 the patient relapsed with increased eosinophils in the PB (24%). Hydroxyurea was started. After 1 year the skin infiltration was still present, but the patient presently has mild eosinophilia and a satisfactory general condition.

Case #5 (MS). A 56-year old woman was admitted in June 1996 with a 2-month history of eosinophilia, associated with an erythematous rash and fever. On admission hyperleukocytosis (44 × 10^9/L), and hyper-eosinophilia (48%) were present. Therapy with methylprednisolone obtained a satisfactory reduction of PB eosinophils: ever since the patient has been receiving intermittent courses of steroid therapy with persistent mild-to-moderate eosinophilia.

Case #6 (GaL). A 38-year old man presented in May 1994 with fatigue, fever, weight loss, hepatosplenomegaly, hyperleukocytosis (62 × 10^9/L), and hyper-eosinophilia (48%). Therapy was started first with methylprednisolone and hydroxyurea, obtaining partial control of the PB count. In August 1996, the patient had 19.2 × 10^9/L WBC and 50% eosinophils, interstitial lung infiltration, myocardial fibrosis with decreased cardiac ejection fraction; in addition, he suffered a transitory ischemic attack. In September 1996 the patient developed severe anemia and thrombocytopenia, and supportive therapy with blood and platelet transfusions was instituted. In October 1996, the patient was put on interferon therapy (IFN-α at 3 MU/day). A satisfactory clinical and hematologic control of the disease was obtained after 8 weeks. The patient is presently receiving IFN-α treatment at a dose of 3 MU 3 times weekly and is in a good condition with a WBC 5 × 10^9/L and 18% eosinophils.

### Cytogenetics
Bone marrow cells were cultured for 24 and 48 hours in RPM I medium to which 10% fetal calf serum was added. The cytogenetic procedure included mitotic block by colcemid, hypotonic shock in 0.075M KCl solution and fixation in 3:1 methanol/acetic acid solution. A minimum of 20 metaphases was added. The cytogenetic procedure included mitotic block by colcemid, hypotonic shock in 0.075M KCl solution and fixation in 3:1 methanol/acetic acid solution. A minimum of 20 metaphases was analyzed for each case: karyotype aberrations were described according to the ISCN.5

### Interphase cytogenetics
FISH studies were performed on cells taken from the same samples that were used for the cytogenetic analyses. The following commercially available probes were tested in all patients: the 13q14 probe for the Rb locus, the p53.3 cosmid recognizing p53 gene sequences at the 17p13 chromosome band, and a probe located at 5q31, a probe hybridizing to 7q31.1 sequences (probes provided from Vysis®, IL, USA). To confirm the presence of a 3q25-q27 deletion and of +11, a 3q27 probe (a gift from Françoise Birg, Institute of Human Genetics, Marseille, France)
and a chromosome 11 specific probe (Vysis®) were used in two patients. The 13H4 YAC probe encompassing the region at 11q23, where MLL gene breakpoints are located, was prepared by B. Young (Cytogenetics Laboratory, St. Bartholomew's Hospital Medical College, Department of Medical Oncology, London, United Kingdom) and tested in all patients. The probes were biotinylated, or digoxigenin-labeled and tested in 5 normal control samples. To detect the presence of a BCR/ABL fusion gene a commercially available probe set was used consisting of a BCR probe of approximately 300 kb in size, which was directly labeled with SpectrumGreen fluorophore and an ABL probe of 200 kb in size, directly labeled with SpectrumOrange fluorophore (Vysis®).

The cut-off point for recognizing a case as Ph-positive was set at the mean percent value of cells exhibiting a fusion signal that was observed in normal controls, plus 3SD (0.748% + 3SD / 0.564% = 2.44%).

Hybridization using the 5q31 probe on PB smears was performed as previously described6 in one patient to be able to correlate FISH results and cell morphology.

Results

No chromosome abnormalities were detected by conventional cytogenetic analysis in 4 patients; interphase FISH did not reveal BCR/ABL fusion or cryptic deletions at 5q31, 7q22, 11q23, 13q14. In two patients (cases #1 and 4) clonal karyotype abnormalities were seen: patient #1 had a del(3)(q25q27)(3); 46, xy[17] - - - - - 28% -

Discussion

Few data are available on the cytogenetic profile of HES. We were able to collect 23 cases with a clonal anomaly in a literature review summarized in Table 3. In this study, two previously unreported aberrations, namely del(3q) and +11 plus +14 in two unrelated clones, were detected by chromosome analysis and by interphase FISH. In addition a cryptic 5q31 deletion was shown to occur in cells of the eosinophilic lineage and in a fraction of granulocytic cells in the patient with +11 and +14. The difference observed between CCA and FISH in estimating the size of the abnormal clones deserves some comments, because at least 2 factors may account for these discrepancies: a) difference in the in vitro mitotic index of distinct cell lineages may influence the abnormal-to-normal metaphase ratio; b) the relatively low number of karyotypes available for analysis.

Deletions of the long arm of chromosome 3 are an infrequent event in myeloid disorders, having been found sporadically in myelofibrosis with myeloid

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Table 2. Karyotypes and FISH results.

<table>
<thead>
<tr>
<th>Pts.</th>
<th>Karyotype [no. of cells]</th>
<th>FISH analysis (% of cells with deletion/rearrangement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL</td>
<td>46, xy, del(3)(q25q27)(3); 46, xy[17]</td>
<td>- - - - - 28% -</td>
</tr>
<tr>
<td>MO</td>
<td>46, xy[20]</td>
<td>- - - - - - -</td>
</tr>
<tr>
<td>BA</td>
<td>46, xy[20]</td>
<td>- - - - - - -</td>
</tr>
<tr>
<td>RA</td>
<td>45, xy-21[3]; 47,xy+14[2]; 47,xy+11[1]; 46,xy[14]</td>
<td>- 20% - - - - -</td>
</tr>
<tr>
<td>MS</td>
<td>46, xy[20]</td>
<td>- - - - - - -</td>
</tr>
<tr>
<td>GL</td>
<td>46, xy[20]</td>
<td>- - - - - - -</td>
</tr>
</tbody>
</table>

*= not significant, below the cut-off point for positivity described in the text.

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Figure 1. An eosinophil cell with gross cytoplasmic granules and a bilobed nucleus showing 1 signal after hybridization with the 5q31 probe; a neutrophil with two 5q31 alleles is shown on the left.
metaplasia and in few cases of myelodysplasia. Our patient (#1) had a morphologically normal BM, except for the eosinophilic component and did not show peripheral cytopenia or BM fibrosis throughout the course of his disease.

Structural rearrangements involving the 5q31 region were previously found in HES/CEL and the existence of a possible correlation with deregulated expression of the \( \text{IL5} \) gene mapping at this region was postulated. Unlike previous cases our patient did not show a 5q31 translocation making it unlikely that a juxtaposition of \( \text{IL5} \) to other gene promoters may have been a mechanism accounting for eosinophil proliferation in this case. Though deletions at 5q31 as well as +11 and +14 were previously detected in myelodysplastic syndromes and acute myeloid leukemias, no evidence for dysplastic maturation of any bone marrow cell lineage was found in our patient (#4), either at presentation or during the course of the disease.

FISH is a sensitive method which was shown to allow detection of submicroscopic rearrangements in myeloid as well as in lymphoid neoplasias; thus the presence of cryptic DNA losses in patients with MDS or chronic lymphocytic leukemia or non-Hodgkin's lymphomas was documented in a substantial portion of cases. Despite the use of a panel of probes detecting chromosome lesions frequently occurring in a spectrum of myeloid neoplasias, we did not identify these rearrangements in 4 of our patients with a normal karyotype. This finding suggests that these patients either had a reactive condition or that other as yet unknown chromosome defects may underlie the development of HES. Interestingly, 2 of these cases (#3 and 6) lacking detectable chromosome defects suffered aggressive disease, with disordered hematopoiesis and multi-organ involvement in one and rapidly fatal disease with prominent splenomegaly in the other patient. Conversely, a more benign course was observed in the two cases with chromosome abnormalities, suggesting that the presence of chromosome changes may not necessarily be an indicator of aggressive disease in HES.

In conclusion, our data suggest that the presence of an abnormal karyotype is not a frequent event in HES and that the variable chromosome anomalies found in this condition mirror the heterogeneity of clinical manifestations. More cases need to be studied in order to identify clinically significant chromosome changes in this rare hematologic disorder.

Contributions and Acknowledgments
GLC, AC, RB, were responsible for the conception of the study, its design, and data handling. RB, MGR, RM, AB, FC, CM, performed the cytogenetic and FISH analyses. All the authors contributed to the analysis and writing of the paper. The authors are listed according to the importance of their contribution to the work. The last name is that of the principal clinician involved and the senior author.

Disclosures
Conflict of interest: none.
Redundant publications: no substantial overlapping with previous papers.

Manuscript processing
Manuscript received September 14, 1999; accepted February 8, 2000.

Potential Implications for clinical practice
- Different chromosome defects are associated with development of hypereosinophilic syndrome.

References


