CORRESPONDENCE

We therefore postulated that the high frequency of MDR1 expression might be one reason for the poor response of elderly AML patients to therapy. In contrast to the high frequency of MDR1 expression found by us, Venditti et al found that only 54% of their elderly patients had MDR1+ blasts, a frequency similar to the 40% of younger patients they found to be MDR1+.

Both biologic and methodologic differences may account for the differences between the findings of our studies and those of Venditti et al. First, the patient populations studied may be different: the 211 patients we studied included some with secondary disease, whereas Venditti et al examined only de novo disease. Secondly, and surprisingly considering their patients all had de novo AML, Venditti et al’s patients had a much higher frequency of unfavorable cytogenetics (58%) than that found in our study (32%). This frequency of unfavorable cytogenetics is significantly higher than what has been reported by several other groups.1,3,4 Thirdly, methodologic differences to detect MDR1 likely also play a critical role in the differences in frequency of MDR1 expression detected. Venditti et al used the cross-reactive antibody C219 to detect MDR1 expression. C219 is less specific for MDR1 than MRK16, because it cross-reacts with the related protein, MDR2, and various other proteins.3,5 In addition, because C219 is directed against a cytoplasmic epitope of MDR1, technical consistency may be more problematic than when an antibody directed against a surface epitope is used.6 Although Venditti et al state that C219 and MRK16 staining were highly correlated when compared on a subset of 103 patients, it is unclear how the correlation was or that MRK16 in their hands gave the same expression data as in ours.1,2 A second methodologic factor may be the sensitivity of the assay; we use a duochrome reagent system to augment the often weak expression signal in primary leukemic specimens.2 This step is essential to detect many MDR1+ primary AML samples; indeed, in our experience, without this reagent, many false-negative cases result. Lastly, we analyze the data using the KS statistic, rather than the percentage of positive cells, because we have found this, in our hands, to be more reliable in analyzing dimly positive cell populations. Such methodologic differences are well recognized and can lead to differences in results between laboratories.2,4 They highlight the need for detailed descriptions of procedures to detect MDR1 and of using more than one assay (eg, functional studies) to better detect MDR1.2

Venditti et al found that MDR1 expression was similar among both younger and elderly patients with AML. Their experience is quite different from ours; in our recent studies on MDR1 expression among younger AML patients (median age, 43 years) using similar methodologies, we found that only 35% of patients were MDR1+, in contrast to the 71% found in our elderly patient group. In addition, unlike Venditti et al, we found that MDR1 expression frequency increased with patient age (P = .01).9

Despite these differences in results of MDR1 frequency, it is interesting that Venditti et al’s studies also confirm the prognostic importance of MDR1 expression to clinical outcome in AML. Venditti et al’s observations that CD7 and TdT expression appear to be phenotypic markers of AML cases with poor prognosis features such as unfavorable cytogenetics and MDR1 expression are interesting. Analysis of expression of these markers may thus be a useful initial indicator of unfavorable prognosis, pending the more lengthy cytogenetic analysis. Use of these markers to predict for MDR1 expression would be less helpful, because analysis of MDR1 expression and function can be performed quite rapidly with the rest of the immunophenotyping profile.

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REFERENCES


A Case of Wiskott-Aldrich Syndrome With Dual Mutations in Exon 10 of the WASP Gene: An Additional De Novo One-Base Insertion, Which Restores Frame Shift Due to an Inherent One-Base Deletion, Detected in the Major Population of the Patient’s Peripheral Blood Lymphocytes

To the Editor:

Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized clinically by the triad of thrombocytopenia, recurrent infections due to defects in the immune system, and severe eczema. The gene responsible for WAS (WASP gene) was recently cloned.1 Since then, large numbers of mutations have been reported in WAS patients with variable clinical phenotypes,2 and it has also been shown that

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substitution of the original sequence. The asterisk denotes the stop codon.

X-linked thrombocytopenia (XLT) resulted from a mutation of the same gene.3

We performed mutation analysis of a boy with WAS and found dual mutations in exon 10 of the WASP gene. The patient died before the present study; thus, only genomic DNA derived from his peripheral blood lymphocytes was available. Genomic DNA, and each exon of the WASP, including flanking introns, was amplified by polymerase chain reaction (PCR). Primers used and each PCR condition were described elsewhere.4 Amplified fragments were purified and directly sequenced using an ABI PRIZM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, CA) and an automated ABI 373A DNA sequencer. The conditions used for the sequencing reaction were also described before.5 To avoid PCR-generated artifacts, we performed sequencing at least twice using a different set of all PCR products in both strands.

The patient studied had two elder brothers who had been diagnosed as WAS based on their clinical and laboratory findings. They died from severe pneumonia and septicemia with bleeding episodes at 10 months of age and 47 months of age, respectively. At 4 years of age, the patient died from unpredictable intracranial hemorrhage; meanwhile, we obtained his peripheral blood lymphocytes and kept them frozen. After the patient died, the couple had a daughter. Genomic DNAs from his peripheral blood lymphocytes was available. Genomic DNA was purified, and sequencing studies of the PCR fragments of the patient’s mother and sister showed that both were carriers for the disease. They had the mutant WASP allele together with the normal allele, and the mutant allele included only the G− mutation. The results of the cloning and sequencing experiment of their PCR fragments were shown in Table 1.

From these results, we speculated the origin of the patient’s dual mutations; the one-base deletion mutation (G−) was derived from his mother, and the other one-base insertion mutation (A+) was generated de novo. The results of the cloning studies suggested that de novo mutation occurred after fertilization, possibly at some level of hematological progenitor. It was shown that the patient’s clones consisted mostly of the dual mutations, with very minor clones having only the G− mutation. PCR-generated artifacts were very unlikely, because 6 different PCR products showed the same results.

Deduced amino acid sequence in both the mutants were shown in Fig 1. In comparison with the single mutation G−, the changes of amino acid in the dual mutation were restricted to the position of 356-356 (Fig 1). The area involved 10 amino acids, and actually 7 amino acids were replaced. Thus, although we could not evaluate the two mutant WASP protein functions, the defect in the case of the dual mutations was considered to be milder than that of the single mutation. A similar case with dual mutations in the WASP gene was recently reported as mild clinical phenotype,6 however, the origin of the dual mutations was not discussed.

Although it is very rarely detected, an additional de novo mutation can reverse severe clinical phenotype in some genetic disorders. Such cases of adenosine deaminase deficiency and atypical X-linked severe combined immunodeficiency were reported, respectively,7,8 In the present case, the additional de novo mutation might change a severe type mutation to a milder one. However, we could hardly confirm the definite difference in clinical severity between the patient and his brothers, who were thought to have only the G− mutation. We thought that the additional mutation was not enough to restore full function of the WASP, or it would take more time to have a definite effect on the phenotype.

The defect of WASP in hematological cells resulted in a growth disadvantage at the stem cell level.9 The present study indicated that there should be a grade of disadvantage depending on the residual

<table>
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<th>Table 1. Cloning Studies of the Individual WASP Allele</th>
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<td><strong>No. of Clones Examined</strong></td>
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<tr>
<td>Patient</td>
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<td>Sister</td>
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*Obtained from 3 independent PCRs.

![Fig 1. Comparison of amino acid sequence from Glu311 to the C terminal part of the each WASP allele. The underlined characters indicate substitution of the original sequence. The asterisk denotes the stop codon.](www.bloodjournal.org)
WASP functions. The lymphocytes with the dual mutations apparently had an advantage over the cells with the single mutation in terms of cell growth in vivo. These findings encourage future attempts of gene therapy for WAS. Introduction and expression of the normal WASP gene even in small numbers of hematological stem cells could be enough to obtain clinical benefit in gene therapy for WAS patients.

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REFERENCES


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c-kit Activating Mutations and Mast Cell Proliferation in Human Leukemia

To the Editor:

Activating mutations of c-kit proto-oncogene have been causally related to neoplastic transformation of mast cell lineage. The first evidence in humans was found in mast cell leukemia cell line HMC-1 with the detection of two mutations in codons 560 (V560G) in the juxtamembrane domain and 816 (D816V) in the cytoplasmic domain of c-kit, resulting in ligand-independent activation of the c-kit product. The predominant effect of D816V could be established by functional studies that demonstrated its pathogenetic role in mast cell transformation. Confirmation of this finding soon came with the identification of D816V in transformed mast cells from a patient with aggressive mastocytosis and lymphocytes from patients with mastocytosis and an associated hematological disorder. The recent identification of a nearby mutation D820-G in aggressive mast cell disease points to the fact that c-kit exon 17 and in particular codon 816 is a hot spot for activating mutations (Fig 1). A puzzling question raised by these studies is whether c-kit activating mutations have only a primary role in mast cell transformation, as described, or whether they may also be a secondary event occurring in hematopoietic cells other than mast cells. Bone marrow mastocytosis in AML-M2 with t(8;21) has recently been described as a new characteristic association of unknown pathogenetic significance. Previous findings by Valenti et al defined a unique form of myeloblastic transformation in myelodysplasia and chronic myeloid leukemia characterized by spontaneous factor (MGF)-independent growth/differentiation of mast cells. Although the investigators could not provide any molecular evidence for their observations, they suggested that there might be a cellular MGF-independent defect (a point mutation in the MGF or MGF-receptor/c-kit genes) leading to evolution of mast cells from a malignant clone. We believe this rationale can be supported by the unique case we are reporting on here. We have identified a novel c-kit mutation (D816Y) in peripheral blood cells from a patient with acute myeloid leukemia (AML) of the M2 subtype, characterized by the massive presence of mast cells in bone marrow and rapid progression of the disease. The mutation, a G → T transversion at nt 2467 of the c-kit gene resulting in Asp816→Tyr substitution (Fig 1), corresponds to the D814Y mutation identified and characterized in the murine P815 mastocytoma cell line. Stem cell factor (SCF) transcripts were not detected by reverse transcription-polymerase chain reaction in leukemic blasts from bone marrow and peripheral blood from the patient. This finding indicates that the massive mast cell growth and differentiation observed in the patient’s bone marrow is not dependent on SCF stimulation. Thus, the c-kit activating D816Y mutation leads to independent SCF growth like its murine D814Y counterpart also in humans.

Cytogenetic analysis on the patient’s blasts showed a 47, XY t(8;21) +4 karyotype in all the metaphases analysed. The concomitance of two AML-specific chromosomal changes and D816Y kit mutation raises the question of their timing and role in the onset and evolution of leukemia in the patient under study. Translocation t(8;21) has proved to play a primary role in M2 acute myeloid leukemia and the blast immunophenotype clearly pointed to this event being a primary one in our case. Based on the cytofluorimetric finding of a subset of CD117 blasts (23%) in our patient, we argue that the D816Y mutation was a
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