The Myeloperoxidase Gene in Acute Promyelocytic Leukemia

Acute promyelocytic leukemia (APL) is a subtype of leukemia with a specific cytogenetic translocation [t(15;17)(q22;q11.2)]. These cells express abundant myeloperoxidase, an enzyme that catalyzes the synthesis of hypochlorous acid, which contributes to the microbicidal function of granulocytes.

Weil *et al.* (*1*) report the mapping of the myeloperoxidase gene (*MPO*) to human chromosome 17(q12–q21), the region of the breakpoint on chromosome 17 in APL. The chromosomal localization of *MPO* at bands q11–q21, on the basis of in situ hybridization data, is in disagreement with two published studies mapping *MPO* to chromosome 17 on bands q21–q23 or q22–q24, several million base pairs away from the breakpoint of APL on chromosome 17 (*2, 3*). Chromosomal localization by in situ hybridization is generally less accurate than screening a panel of somatic cell hybrids containing fragments of chromosome 17. Using such a panel, van Tuinen *et al.* ruled out localization of *MPO* to 17q12–q21 (*2*).

In support of the more distal location of *MPO* on 17q reported by both van Tuinen *et al.* (*2*) and Chang *et al.* (*3*), we now know that a number of genes intervene between the APL breakpoint and the *MPO* locus. Loci for *NGFR* (*4*) *EMOB3* (*5*), *HOX2* (*6*) and *GFAP* (glial fibrillar acidic protein) mapped by B. Westermark and colleagues in collaboration with one of us (K.F.H.) are distal to the APL breakpoint but proximal to the *MPO* locus (*7*). The observation by Weil *et al.* (*1*) that *MPO*, normally located on chromosome 17q, was translocated to chromosome 15 in APL is therefore not surprising and supports the conclusions of others (*2, 3, 8*).

Further, Weil *et al.* (*1*) present evidence from Southern blotting of rearrangement of *MPO* in DNA from bone marrow of two of four patients with APL. The data suggest a high frequency of rearrangement of the transcriptionally active *MPO* in APL and could represent an important step in understanding the etiology of the disease.

To determine the incidence of rearrangement of *MPO* in APL, DNA samples from bone marrow of 13 patients with APL were digested with Bam HI and Bgl II, electrophoresed, Southern blotted, and hybridized with several ^{32}P-labeled cDNAs coding for *MPO* (Fig. 1). No novel bands were detected, while all normal bands identified by Weil *et al.* (*1*) were detected. We conclude that rearrangement of *MPO* is at most an infrequent occurrence in APL. DNA from three patients with APL was subjected to inverted field gel electrophoresis after digestion with infrequently cutting restriction endonuclease. In no case could rearragements of the gene be detected within 1000 kb of the coding region.

Because of our inability to confirm the findings of Weil *et al.* (*1*), we examined the characteristics of the two *MPO* cDNA probes used in their study. Their two recombinant plasmids, pHMP10A and pHMP2E, cover an apparent ~2 kb at the 3′ end of the longest (3.3 kb) of the two RNAs that code for *MPO*. These probes have not been sequenced, but the authors state that they have compared their restriction maps with the sequence of the near full-sized clone that we have documented to be *MPO* (*9*). Their restriction map in the 5′ end of clone pHMP10E and in the 3′ end of clone pHMP2E is different from the map that can be derived from the identical *MPO* cDNA sequence data of Johnson *et al.* (*9*) and Morishita *et al.* (*10*) (Fig. 2). The relevant differences are that the Bgl II site at the 5′ end of pHMP10A does not exist in the published sequences; however, a Kpn I site found in this region, as deduced by sequencing data, is missing in their clones. The restriction map of the 3′ half of pHMP2E clone is completely different from the maps deduced by the sequencing data. Furthermore, clone pHMP2E extends 600 base pairs further in the 3′ direction than any reported sequence. We therefore suspect, barring trivial mistakes on our part in generating their restriction map, that Weil *et al.* have either cDNAs that are the result of a cloning artifact or that *MPO* contains an extremely high degree of polymorphism.

Furthermore, the results as presented by Weil *et al.* (*1*) do not prove that *MPO* is rearranged in APL for several reasons: (i) In one of their APL samples (L2), they found an extra band using only one of three restriction enzymes (Bgl II), which does not rule out an infrequent restriction fragment length polymorphism (RFLP). The extra Bgl II sites present in their cDNA clones, if real, would suggest an RFLP. (ii) The genomic restriction map of *MPO* (*11*) suggests that the rearrangements which introduced new sites for Bgl II in one of their samples

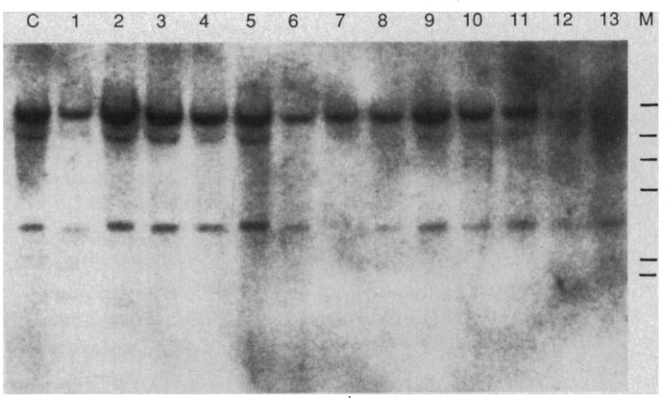

Fig. 1. Restriction pattern of *MPO* in DNA of bone marrow cells from 13 patients with acute promyelocytic leukemia and DNA of normal human fibroblasts (C). The DNA from patients was derived from a population of cells that contained more than 80% APL cells. The leukemic cells of each patient contained the typical t(15;17) of APL. Samples were digested with Bgl II and probed with the Kpn I–Hind III 0.5-kb fragment of pMP02 (*9*), a 3′ specific probe. Digestion, electrophoresis, Southern blotting, and hybridization were performed by standard techniques (*10*). Gel-purified fragments were ^{32}P-labeled by random priming, to 10^9 cpm/μg (*11*). The same DNAs were also digested with Bam HI and hybridized sequentially with the same probe and with ^{32}P-labeled pMP062, a full-length cDNA (*9*). The restriction pattern of DNA samples from the patients was identical to that from normal fibroblasts.

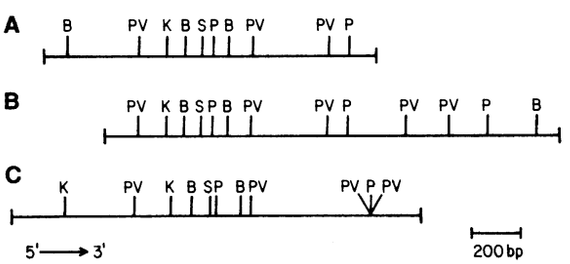

Fig. 2. Restriction maps of putative *MPO* cDNA clones. Maps (**A**) and (**B**) are those reported by Weil *et al.* (*1*). Map (**C**) is computer generated from the 3′ region of cDNA sequence data of Johnson *et al.* (*9*) and of Morishita *et al.* (*10*). Restriction endonuclease cleavage sites are Bgl II (B), Kpn I (K), Pst I (P), Pvu II (PV), and Sma I (S).

and for Hind III and Bgl II in the other would affect the size of other restriction fragments in their restriction digests. These were not observed. (iii) One or several novel restriction fragments occurred in the DNA from one of their APL patients with an *MPO* rearrangement (L1) when the patient was in remission; theoretically the restriction pattern should have been normal or at least the same as the pattern during active disease.

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Acute promyelocytic leukemia (APL) is consistently associated with a reciprocal translocation involving chromosomes 15 (band q22) and 17 (band q11–q12) (*1*). The DNA segments directly involved in this translocation, t(15;17), have not yet been identified and, as a result, its role in the pathogenesis of APL is not yet determined.

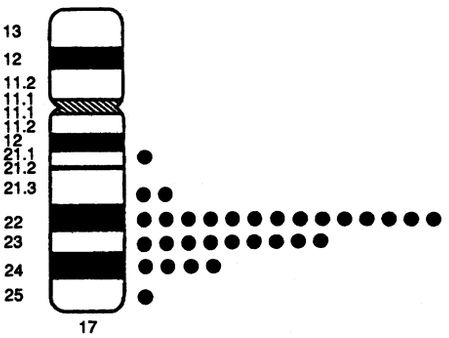

Fig. 1. Distribution of *MPO*-hybridizing sites on normal human chromosome 17. In the 105 metaphases analyzed, 21% of 148 grains were observed on the long arm of chromosome 17 ($P < 0.001$) with 75% of grains on band 17q22-q23.

Weil *et al.* (*2*) report that the myeloperoxidase gene (*MPO*) maps to human chromosome 17(q12–q21) and that it is rearranged and translocated to chromosome 15 in cases of APL. The implications of these results, if correct, are important to an understanding of the pathogenesis of APL, as they provide precise information about the location of the breakpoint on chromosome 17 and indicate the molecular strategy for identifying critical DNA sequences on the recombinant chromosome 15.

As part of more extensive research aimed at molecularly characterizing the t(15;17), we also mapped *MPO* to normal human chromosomes and determined the position of the breakpoint on chromosome 17 with respect to *MPO* in cases of APL. However, our findings do not agree with those reported by Weil *et al.*

For chromosomal localization of *MPO*, we used a nearly full-length cDNA probe representative of the human *MPO* gene [clone pMPO62, a gift from G. Rovera; see (*3*)]. Labeled pMPO62 DNA was hybridized to chromosome preparations from normal peripheral blood lymphocytes. The *MPO* probe hybridized to chromosomes 17, and silver grains were consistently located on band q22–q23, as has been previously reported (*4*) (Fig. 1). We conclude that *MPO* maps to a region distal to the chromosomal region 17q11–q12 involved in the t(15;17).

To investigate whether parts of the *MPO* gene or its immediately flanking sequences were directly affected by the breakpoint on chromosome 17, we performed Southern blot analysis of genomic DNA from 27 cases of APL. Diagnosis of APL was formulated according to established clinical, morphological, cytochemical, and immunophenotypic criteria in all cases. Cytogenetic data, which were available for 13 cases, revealed the presence of a typical t(15;17) in all cases. DNAs were digested with Bam HI, Bgl II,

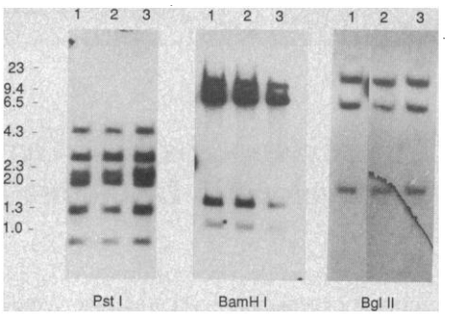

Fig. 2. Analysis of the genomic organization of the *MPO* locus in cases of APL. DNAs isolated from normal peripheral blood lymphocytes (lane 1) and from bone marrow cells from APL patients (lanes 2 and 3 for two representative cases) were digested with the indicated restriction enzymes and hybridized with the MPO62 probe. The sizes of molecular weight markers are given in kilobases.

Pst I, and Kpn I and were hybridized to the pMPO62 probe. This combination of restriction enzymes and probe allowed the entire *MPO* locus to be explored plus an area approximately 5 kb upstream from the putative 5′ end and downstream from the polyadenylation signal. Weil *et al.* also used Bam HI and Bgl II restriction enzymes to detect *MPO* rearrangements in cases of APL. In our study, none of the 27 APL cases diverged from the normal restriction enzyme pattern as determined in 21 normal DNAs (Fig. 2). We therefore conclude that the breakpoint on chromosome 17 of the t(15;17) was not located within a measurable distance from *MPO* in any of the 27 cases of APL studied.

Although we are unable to offer a definitive explanation for the apparent contradiction between our results and those of Weil *et al.*, we note that the restriction map of the *MPO* cDNA probe they used in both the in situ hybridization and Southern blot experiments [probe pHMP10A (*2*)] differs significantly from the one we used (*3*) and from another that has been reported (*5*) at the 5′ end. This heterogeneity suggests (i) that the general population is genetically polymorphic for the restriction enzyme recognition sequences (Bgl II; Kpn I), which differ in the *MPO* cDNAs mentioned above, or (ii) that the pHMP10A and pMPO62 clones hybridize to different genomic fragments because of *MPO*-related genes or alternative splicings present in the processing of immature *MPO* RNA. However, we found no evidence of Bgl II or Kpn I restriction enzyme polymorphisms in 21 normal DNAs when pMPO62 was used as the hybridizing probe, and the genomic fragments identified by the pHMP10A probe appeared also to be detected by the pMPO62 probe with the use of restriction enzymes Bam HI, Bgl II [Fig. 2 and (*2*)], and Hind III. The publication of

the nucleotide sequence of clone pHMP10A should help to clarify these controversial issues.

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Response: We reported the chromosomal localization and the translocation and rearrangement of the myeloperoxidase gene (*MPO*) in two patients with acute promyelocytic leukemia (APL) (*1*). This report has caused some concern among other groups who have localized the *MPO* gene to a more distal site (*2, 3*) on chromosome 17 and among those who have not demonstrated any molecular rearrangements of this gene using two different, independently isolated, *MPO* cDNA clones (*4*).

We too are eager to resolve these discrepancies. With regard to the chromosomal localization of *MPO*, in situ hybridization of the pHMP10A clone resulted in specific labeling of chromosome 17 at bands q11–q22. Hybridization of this clone and of the pHMP2E probe to metaphase cells from bone marrow aspirates from APL patients resulted in specific labeling of the normal chromosome 17 at bands q11–q22 and of the translocation derivative 15q+ at bands q12–q21. We concluded from these studies that *MPO* was located at q12–q21, distal to the breakpoint of the t(15;17) at band 17q11.2, and that it had been relocated to chromosome 15 as a result of this translocation. The translocation of *MPO* to the derivative 15q+ in an APL patient has also been shown by Liang *et al.* (*5*). As noted by Miller *et al.*, in situ hybridization experiments conducted by other investigators resulted in specific labeling of a more distal site on chromosome 17 at bands q22–q23 (*2, 3*); however, the localization of *MPO* to chromosome 17 with the use of a panel of somatic cell hybrids containing deleted or translocated segments of this chromosome resulted in the assignment of *MPO* to 17q21–q23 (*3*), which overlaps with our localization. Van Tuinen *et al.* (*3*) found that a hybrid which contained bands 17q21 to qter (including most or all of 17q21, as illustrated in figure 1 of their paper) was positive for *MPO* sequences. Discrepancies in the localization of genes to specific chromosome bands by investigators who use different techniques or different probes are not uncommon. It is possible that the more distal localization of *MPO* to chromosome 17 is correct (*2, 3*), in which case rearrangements of *MPO* in APL cells would likely be unrelated to the translocation breakpoint. This possibility was clearly stated in our report. Close proximity of *MPO* to the t(15;17) breakpoint is not requisite for rearrangements of this gene to occur. If *MPO* is located at 17q22–q23, why our probe hybridized to the adjacent proximal bands of this chromosome is unclear. Additional hybridization with the use of our *MPO* clones and other *MPO* probes may clarify this issue.

Regarding the rearrangements, our finding has been extended for one of the APL patients in our study from whom a lambda genomic library has been constructed. An abnormal clone has been isolated and characterized from the library of patient L2, whose bone marrow DNA exhibits a novel 3.1-kb Bgl II band in Southern blotting with our *MPO* clone, pHMP10A. Restriction enzyme mapping of this clone reveals extensive homology with a normal *MPO* genomic clone, except for a novel Bgl II site in which two Bgl II fragments of 3.1 and 4.5 kb replace the normal 7.6-kb fragment. Only the 3.1-kb and normal 7.6-kb fragments hybridize to our *MPO* cDNA probe. Both of the novel Bgl II fragments localize to chromosome 17 by somatic cell hybrid panels, and preliminary sequence data indicate that the novel Bgl II site may be the result of a single base change. Whether this represents a leukemia-related point mutation or a rare constitutional restriction fragment length polymorphism cannot be determined at this time because material from patients in remission is unavailable. We have, however, shown that restriction fragment length polymorphism is not present with Bgl II or Bam HI restriction enzyme recognition sequences (*6*). This is corroborated by the data presented by Donti *et al.*

We agree that rearrangement of the *MPO* gene in APL is a rare occurrence. Since publication of (*1*), 15 additional APL patients have been studied by Southern blot analysis. Fourteen of them showed no novel Bam HI or Bgl II fragments. One, however, showed a novel 18-kb Bgl II fragment, as well as a novel Bam HI doublet in the 5.5- to 6.0-kb range. None of these fragments was present when remission DNA from the same patient was studied. Thus, if patient L2 is omitted, two out of 19 M3 patients analyzed in this laboratory with the use of *MPO* clone pHMP10A as a probe showed novel leukemia-associated bands that disappeared upon remission. Our cDNA did not hybridize to any genomic fragments in normal or HL-60 DNA that were different from those reported by others. These recent data help resolve some of the questions raised, especially by Miller *et al.*, and suggest that the discrepancies noted are due to differences in patient material rather than in cDNA clones.

Regarding our cDNA clones, only pHMP10A was used for Southern blotting. We deliberately chose not to use pHMP2E for the exact reason mentioned by Miller *et al.*, that is, it extends 600 base pairs further 3′ than accountable by *MPO* coding sequences. This was not discussed at length in our paper. We have recently remapped clone pHMP10A. When compared with the map from Miller *et al.*, this clone actually ends just 3′ to the Kpn I site and does not contain the Bgl II site in question. Why earlier mapping experiments revealed this Bgl II site remains unclear. We can conclude, however, that clone pHMP10A is virtually identical to other published *MPO* cDNA sequences (*4, 7*). We believe it is important to reiterate one of the main points of our paper: two novel restriction fragments seen in leukemic (L1) DNA (with the use of Bam HI and Bgl II, not Hind III, as stated by Miller *et al.*) are absent from remission DNA when it is probed with a gene (*MPO*) that is tightly restricted both functionally and temporally within the myeloid lineage. This strongly suggests a leukemia-associated rearrangement. We have no explanation for the weak bands observed in the Southern blot of the remission sample from patient L1. In