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RAPID COMMUNICATION

A Novel Gene, *AF1q*, Fused to *MLL* in t(1;11)(q21;q23), Is Specifically Expressed in Leukemic and Immature Hematopoietic Cells

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Translocations involving chromosomal band 11q23 are associated with leukemias. These translocations fuse the *MLL*, a gene with sequence homology to the *Drosophila trithorax*, to genes from a number of other chromosomal loci. We have characterized two t(1;11)(q21;q23) translocations that fuse the *MLL* gene to a novel gene, *AF1q* on chromosomal band 1q21, in two infants with acute myelomonocytic leukemia (AMMOL). In one of these patients, der(11) represents an in-frame fusion of the N-terminal portion of *MLL* gene to the complete *AF1q* open reading frame, whereas der(1) does not give rise to an open reading frame. This observation suggests that the N-terminal portion of *MLL* gene is critical for leukemogenesis in translocations involving band 11q23. The predicted wild-type AF-1q product is a 9-kD protein with no similarity to any other protein in the data banks. The *AF1q* mRNA is highly expressed in the thymus but not in peripheral lymphoid tissues. In contrast to its restricted distribution in normal hematopoietic tissue, *AF1q* was expressed in all leukemic cell lines tested.
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SPECIFIC CHROMOSOMAL translocations play an important role in oncogenesis of various malignancies, including distinct types of hematopoietic malignancies.[1] Chromosomal translocations lead to malignancy mainly by one of two mechanisms. The first is transcriptional activation of a putative oncogene. This mechanism has been documented in many lymphoid malignancies where T-cell receptor and Ig genes participate in various translocations. Activation of the oncogenic potential in these cases is the result of juxtaposing regulatory elements of T-cell receptor and Ig genes near an oncogene causing transcriptional activation.[2] The second mechanism is the generation of transforming fusion products.

Chromosomal translocations involving the 11q23 region belong to the second category. However, this region is unique in that it is involved in translocations with several other chromosomal loci resulting in various types of leukemias.[3-5] Frequent translocations of the 11q23 band have been described with at least 15 different chromosomal partners.[5] This chromosomal band is frequently rearranged in acute lymphoblastic (ALL), in acute myelomonocytic (AMMOL), acute monocytic (AMOL), and acute myeloid (AML) leukemias mostly in reciprocal exchanges with specific translocation partners. The tissue specificity of the leukemia depends very much on the particular gene translocated to the 11q23 locus. Thus, the t(4;11)(q21;q23), t(11;19) (q23;p13), t(11;x), (q23;q13), and 1(1;11) (p32;q23) are found in 10%, 2%, >1%, and >1% of ALL, respectively.[6] Reciprocal translocation between 11q23 and chromosomal bands 9p22, 6q27, 2p21, 10p11, 17q25, and 19p13 are found in 5% to 6% of AML whereas t(1;11) t(q21;q23) are found in AMMOL.[7]

Recently, the gene involved in the recurring 11q23 chromosomal translocations has been identified.[8-10] This gene, called *ALL-1, HRX* or *MLL*, encodes an unusually large protein of 3,972 amino acids (431 kD) that is homologous to *Drosophila trithorax*, a homeotic transcriptional regulator.[11] The homology between *MLL* and *trithorax* extends over four putative zinc finger DNA binding domains. In addition, the human gene alone contains two other potential DNA binding domains. The first consists of three AT hooks motifs that are implicated in minor groove DNA binding, and the second is a region homologous to mammalian DNA methyltransferases.[12] The translocation breakpoints in all cases studied are tightly clustered in a 9-kb DNA region that spans exons 5 to 10 of the *MLL* gene.[9,13-16] The translocation disrupts the *MLL* gene between the N terminal "AT hook" domains and methyltransferase putative DNA binding domains, and the C terminal zinc finger motifs.

Six different translocated partners fused to the *MLL* gene have been molecularly identified to date; these include 4q21, 9p21-22, 19p13, 1p32, 6q27, and 17q25.[9,10,15,17-19] In addition, partial duplication of the *MLL* gene spanning exons 2 through 6 were found in AML patients with trisomy 11 or without any cytogenetic abnormalities in chromosome 11.[20] Comparison of the amino acid sequence of these six proteins involved in translocation fails to suggest a specific sequence requirement for the fusion partner of band q23. However, it seems that the fusion partners of *MLL* may have in common motifs involved in protein interactions. Four of these proteins, *AF-4, AF-6, AF-9,* and *ENL*, contain domains rich in serine and proline residues, whereas *AF17* contains a leucine zipper motif. Three of these proteins, *AF-4, AF-9,* and *ENL*, contain a nuclear targeting motif as well as other characteristics often found in transcription factors, whereas the other two, *AF-6* and *AF-1p*, are homologous to cytoskeltal or cytoplasmic proteins involved in signal transduction. Thus, these motifs may act by altering the specific interaction regulating the multifunctional transcription activation by *MLL* and thus contribute to its oncogenic activity.

In the present work we report the cloning of the cDNA of the *AF1q* gene, the fusion partner of *MLL* gene in t(1;11)(q21;q23) in infants with AMMOL.

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Submitted October 12, 1994; accepted November 6, 1994.
Supported by a grant from the Canadian National Cancer Institute. W.T. is a recipient of a Terry Fox fellowship of the Canadian National Cancer Institute.
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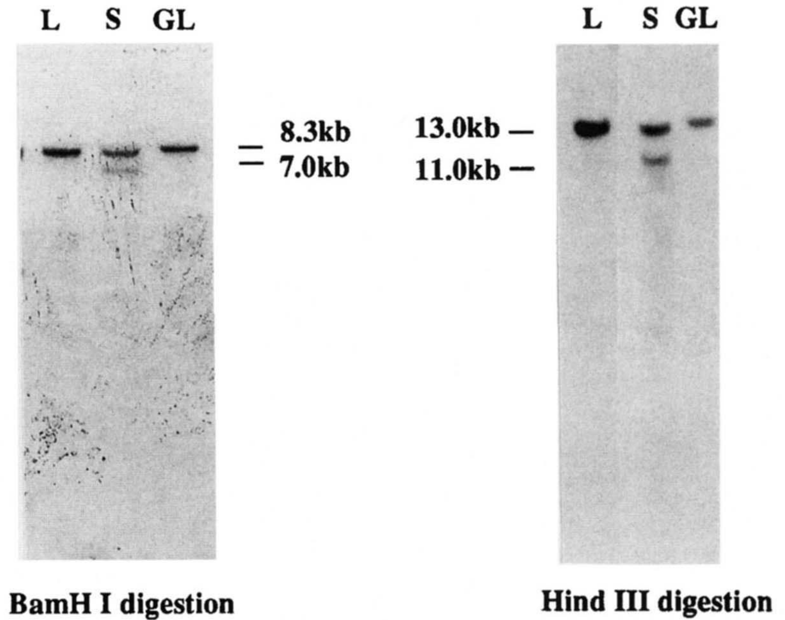

Fig 1. Southern blot analysis of DNA from two patients with t(1;11)(q21;q23). DNAs from leukemic cells of two patients (L and S) with t(1;11)(q21;q23) and a normal control (GL) were digested with *Bam*HI or *Hin*dIII and hybridized with the B859 probe. The germ-line bands are 8.3 and 13 kb for *Bam*HI and *Hin*dIII digestion, respectively; patient S shows translocated bands of 7 and 11 kb which represent the gene involved in this translocation.

MATERIALS AND METHODS

Cell lines of freshly isolated cells and tumor samples. The expression of AF1q mRNA was determined in various human cell lines from hematopoietic tumors. The B1 cell line is a biphenotypic pre-B/myeloid cell line from a leukemic patient with t(4;11)(q21;q23) established in our laboratory.[21] Other tumor cell lines, including the T-lymphoblastoid lines Jurkat, Molt 13, the promyelocytic cell line HL-60, and the monocytic cell line U937, were obtained from American Type Culture Collection (ATCC; Bethesda, MD). Thymic tissue was obtained from samples removed from patients (1 to 4 years old) undergoing cardiac surgery at the Hospital for Sick Children and thymocytes were separated using Hypaque-Ficoll (Quebec, Canada) gradient centrifugation. Patient samples were obtained at diagnosis from bone marrow aspirations of two infants with AMMOL and greater than 90% blast counts.

Southern and Northern blot analysis. High molecular weight genomic DNA was extracted by standard procedures.[22] Cellular RNA was extracted after cell disruption with guanidinium thiocyanate followed by phenol extraction.[23] DNA samples (10 μg) were size

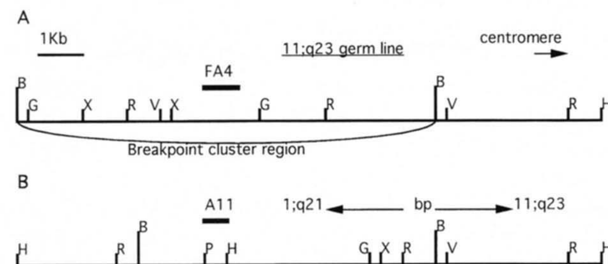

Fig 2. Genomic analysis of the t(1;11)(q21;q23) chromosome translocation. (A) Physical map of the genomic junction of patient S [der(11)]. (B) A map of the corresponding normal region. Fragment A11 was used for screening of cDNA library. B, *Bam*HI; R, *Eco*RI; H, *Hin*dIII; V, *Eco*RV; X, *Xba* I; G, *Bgl*II. The B859 cDNA probe used for Southern blot analysis covers the complete breakpoint cluster region included between the *Bam*HI sites.

cDNA sequence of AF1q:

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AGTCAGCACG GGGGTGCTGG AAGAGATCGG GAATAATAGC GCAGACCAAT   50
GAGCCTAGGG AGATGCTTTC ATCGTCTCTC CTTCCCTCAA GTGTTCTGGA  100
ACCTATCATT TGAATTAGCC GAGTCAGGCA GGAGGGGGCG GGGAATCCTT  150
CCGCCCTTCT TAGGAGGGGC TGCATTGCAG GGGGAGAGTG AACTGACAGA  200
CTCAGTCACT GAAGAGGGAA AAGGAGTGAG AAGACAAAGC CGTCAAAGCC  250
CCAACAGCTT GTATTTCTCC AGCCCGGGGC AGACCCCGGA GCTCCCGAGG  300
CACTCCCTCC ATCTTTGGAA CACGCCAGTA ATTGATTGAT AACAGGAAGC  350

TATGAGGGAC CCTGTGAGTA GCCAGTACAG TTCCTTTCTT TTCTGGAGGA  400
 M  R  D   P  V  S  S   Q  Y  S   S  F  L   F  W  R  M
TGCCCATCCC AGAACTGGAT CTGTCGGAGC TGGAAGGCCT GGGTCTGTCA  450
  P  I  P   E  L  D   L  S  E  L   E  G  L   G  L  S
GATACAGCCA CCTACAAGGT CAAAGACAGC AGCGTTGGCA AAATGATCGG  500
D  T  A  T   Y  K  V   K  D  S   S  V  G  K   M  I  G
GCAAGCAACT GCAGCAGACC AGGAGAAAAA CCCTGAACGT GATGGCCTCC  550
 Q  A  T   A  A  D  Q   E  K  N   P  E  R   D  G  L  L
TTGAGTACAG CACCTTCAAC TTCTGGAGAG CTCCCATTGC CAGCATCCAC  600
  E  Y  S   T  F  N   F  W  R  A   P  I  A   S  I  H
TCCTTCGAAC TGGACTTGCT CTAAGGCCAA GACTTCTCTC TCCCATCACC  650
S  F  E  L   D  L  L   X
TTGCCCTCAT TGTCTTCCCT CTCAAGCCCC TTCCTTTCCA CTCCTTTCCC  700
ATTTTAATCT TGTTCTCTCC CTACTGTGTT GGTGGTGCTG ATGAATCTGC  750
CAGAGTTGAG TTCTATGTAT TTATTTATCT ATCTGTCTAC TCCATTTCTC  800
TCAAAAGCCC TCAAGTCACA AAGTAAATGG TTCAAGCAAT GGAGTACTGG  850
GTCACAGGGA TTCCTCCTTT CCCCCCCAAA TATTAACTCC AGAAACTAGG  900
CCTGACTGGG GACACCTGAG AGTAGTATAG TAGTGCAAAA TGGAAGACTG  950
ATTTTTGACT CTATTATAAT CAGCTTCAGA GATTCCTTAA ACCTTCCTAA 1000
TTTCCTGCTC CAGGGCAGTA AACACAAATA TTTCTTCAAG GGGTGATGAA 1050
AACCTCGGAA GTTTTAATTT GAGGTTATCT GCTACGAAAC AGTATTTCTA 1100
AAAGGCTAAA GTGATAAGTC TCTTGCTTTT TTTTGATCCT GCTCTTATAT 1150
TCTTTTTTTT CCTCAGAGAA ATCAGGAGGG TAGTTAGAGG TATAAAACAG 1200
GAGGAAATAT TATGGAAAAT GAAAATAGGG AAAATAATTG AATCATTTTA 1250
GAAGTAGCTA ATTTCTTTTC TCAAAAGAGT GTCCCTTCTT CACACCTACT 1300
CACTTTACAA CTTTGCTCCT AACTGTGGGT TGAAAACTCT AGCTAAAGAA 1350
AGTTATCAAA TCTTAACATG CATTCCTACT ATTATGATAG TTTTTAAGGT 1400
TTCAATTCAA TCTTCTGAAC GGCATAAGTC CTATTTTAGC CTTACCTCCT 1450
GCATTTGCAA TACGTAATAC TGATCAGTGG GCACAGTTCT TCAGCTACAT 1500
TGAGACCCTG AAATGAACAA TTATATTCTG ACTCGACATC TTGTCCCCAA 1550
TCCTTCCAAA AATATTGATG GTGATTTGTG CTACCATTTA CTCGTTTATT 1600
TaataaaGAC ATTCAATTCC CAAAAAAAA                        1629
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Fig 3. Nucleotide sequence of the AF1q cDNA and predicted amino acid sequence. The fusion point with *MLL* in patient S is shown by the arrow. ATTTA sequences and other AT-rich sequences involved in mRNA degradation are underlined. The polyadenylation signal is italicized.

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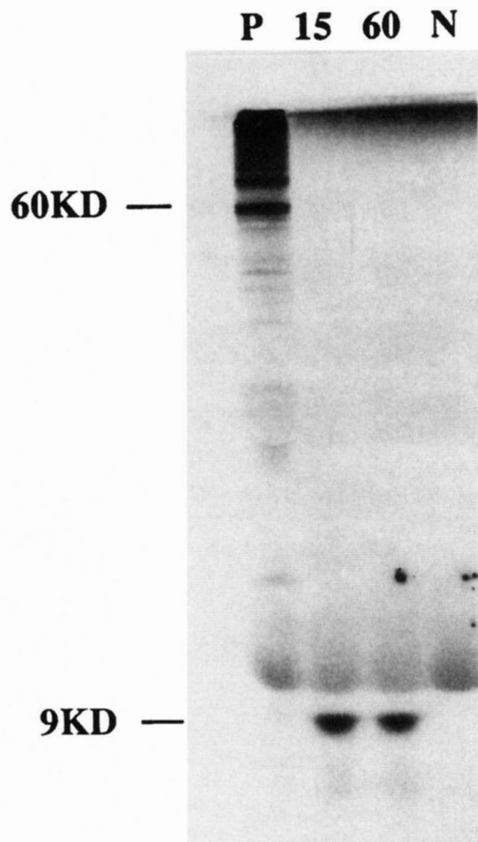

Fig 4. In vitro translation of AF1q cDNA clones. T3-coupled reticulocyte lysate in vitro translation of two AF1q cDNA clones marked 15 (starting at nucletide 350) and 60 (a full-length cDNA clone) and a plasmid control (N).

fractionated on 1.0% agarose gels. The denatured DNA was transferred to Hybond N membranes (Amersham, Arlington Heights, IL). Hybridizations were performed in 0.5 mol/L NaH_2PO_4/Na_2HPO_4, 50% formamide, 1% sodium dodecyl sulfate (SDS), 1 mmol/L EDTA, with radiolabeled probes at 42°C. Northern analysis of multiple human tissues was performed using human tissue mRNA blots obtained from Clontech (Palo Alto, CA).

cDNA and genomic cloning. The rearranged genomic fragments of *MLL* from patient DS with t(1;11)(q21;q23) were cloned into the EMBL-3 phage vector (Promega, Madison, WI) after partial digestion of the DNA with the Sau3A enzyme and size selection. The phage library was screened using a 0.9-kb *Bam*HI fragment derived from the MLL cDNA spanning exons 5 through 11 (obtained from E. Canaani, Jefferson Cancer Institute, Philadelphia, PA[9]). Fibroblast cDNA library was constructed using the oligo (dT) priming cDNA kit (Stratagene, La Jolla, CA). The cDNA library was screened using a repeat-free genomic fragment from the translocated chromosome 1 (labeled A11 in Fig 2). Nucleotide sequencing of both strands was performed by the dideoxynucleotide method.

Polymerase chain reaction (PCR) procedures. Reverse transcriptase-PCR (RT-PCR) was used for semi-quantitative measurements of the expression of AF-1q gene in hematopoietic tissue and cells. First-strand cDNA was synthesized from total RNA, and used as PCR template. Thirty-six cycles were used for amplification of the AF1q cDNA and 23 cycles were used for amplification of actin cDNA. The PCR primers used for AF1q were: VT-1: 5′ GGTGCTGGAAGAGATCGGGA; VT-2: 5′ CAGTGACTGAGTCTGTCAGT, and for β-actin: Actin1: 5′ ATCATGTTTGAGACCTTCAA; Actin2: 5′ CATCTCTTGCTCGAAGTCCA.

Five, 2.5, 1.25, and 0.625 ng total RNA was used in each lane (from left to right) for thymocytes, $CD34^+$ leukemic cells, and Jurkat cells. Tenfold lower total RNA was used for amplification of cDNAs from B1 and Molt13 cells and five times lower concentration of cDNA was used for Daudi cells. The PCR products were then run in 1.5% agarose gel in Tris acetate (TAE) buffer, transferred to nylon membrane, and hybridized with 5′-labeled cDNA-specific deoxyoligonucleotides, (for AF1q PCR: TTCTTAGGAGGGGCTGCATTGCAG and for β-actin: CTGGCATCGTGATGGACTCCGGTGAC), at 42°C. The membranes were washed with 2× SSC/0.1% SDS at room temperature and at 65°C. For the cloning of fused cDNA from a t(1;11)(q21;q23) patient, cDNA synthesis was performed using gene-specific primer. One microgram total RNA was hybridized with antisense primer VT15-1 (3′ of AF1q cDNA: CAAATGCAGGAGGTAA) in hybridization buffer (50% formamide/20 mmol/L PIPES pH 6.4/0.5 mmol/L EDTA/0.2 mol/L NaCl) for 2 hours. First-strand cDNA was made from the hybrid by avian myeloblastosis virus-reverse transcriptase at 42°C for an additional 2 hours. Negative control PCRs were routinely performed by eliminating the cDNA synthesis step; the lack of products in these reactions indicates the absence of genomic DNA contamination. PCR was performed as described above using the following oligodeoxynucleotides: ALL1-5: 5′ATCAGCAAGAGAGGATCCTGC (chromosome 11) and VT15-7: 5′GTCTGCTGCAGTTGCTTG (chromosome 1). PCR products were run in 1.5% agarose gel, transferred to nylon membrane, and hybridized to B859 (MLL cDNA fragment covering all known breakpoints) and VT15 (AF1q cDNA) probes. The PCR products were cloned in pCR II vector (Invitrogen, San Diego, CA) and screened with both B859 and VT15 probes. Positive clones were picked and sequenced.

RESULTS

Involvement of the MLL *gene in t(1;11)(q21;q23).* The *MLL* gene has been shown to be involved in the vast majority of leukemias with 11q23 abnormalities.[16] We used Southern blot analysis to look for translocation at the *MLL* gene locus in two patients with t(1;11)(q21;q23) (Fig 1). A genomic probe B859, which spans exons 5 through 11 of the *MLL* gene, detected a rearranged fragment in both *Bam*HI and *Hin*dIII of DNA digests of one of these patients DNA. These data localized the presumed translocation in this patient within the breakpoint cluster of the *MLL* gene. No translocated fragment is detected in patient L, consistent with previous reports of the existance of 11q23 translocations that involve the *MLL* gene but are outside the breakpoint cluster region.[24]

Cloning of the 1q21 gene fused to MLL. After the localization of the breakpoint in the *MLL* gene, we used the B859 probe, spanning exons 5 through 11 of the *MLL* gene, to clone the genomic translocation from a t(1;11)(q21;q23) leukemic patient. DNA extracted from a bone marrow sample of this leukemic patient at diagnosis was used to construct a genomic library in the EMBL-3 λ phage. Out of 2×10^6 clones screened with this probe, 18 positive clones were isolated. Restriction enzyme analysis of four of these clones

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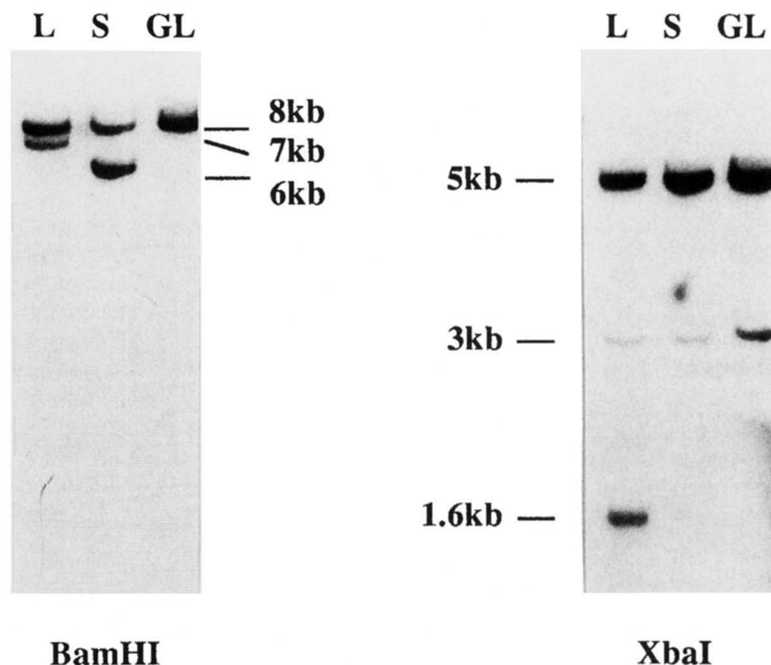

Fig 5. Southern blot analysis of two t(1;11)(q21;q23) patients using chromosome 1 probe. DNA from two patients (L and S) with t(1;11)(q21;q23) and a normal control (GL) were digested with *Bam*HI or *Xba* I and hybridized with the A11 chromosome 1 probe. The germ-line bands are 8 and 5 kb for *Bam*HI and *Xba* I digestion, respectively.

identified a rearranged clone depicted in Fig 2. The apparent breakpoint maps to the intron between exons 6 and 7.

Isolation of the AF1q cDNA. Using a repeat-free genomic DNA segment located 5 kb from the breakpoint on chromosome 1 (A11 in Fig 2), we screened a human fibroblast cDNA library. Nine positive clones were isolated and both strands of the largest clones spanning 1.6 kb and 1.4 kb were sequenced. The sequence of the *AF1q* cDNA presented in Fig 3 contains 1629 nucleotides encompassing an open reading frame of 90 amino acids starting at nucleotide 401. There are stop codons in all frames upstream of the first ATG and a purine at the −3 position in accordance with the consensus for protein initiation. The 3′ untranslated region is highly A + T rich (66%) and contains 15 AT consensus motifs, including four ATTTA, which are commonly found in various cytokine genes and thought to destabilize highly regulated mRNA species.[25] An AATAAA polyadenylation signal starts at nucleotide 1602. The AF1q cDNA does not show any significant homology to any other sequence deposited in the GenBank (Bethesda, MD).

To confirm the predicted open reading frame, we translated two of the AF1q cDNA clones using the T3-coupled reticulocyte lysate in vitro translation system (Fig 4). A single translation product was synthesized and its estimated size (9 kD) is consistent with the deduced amino acid sequence of AF1q. Moreover, the involvement of the *AF1q* gene in t(1;11)(q21;q23) was further confirmed by using the cloned AF1q cDNA as a probe for the detection of rearranged bands in both *Bam*HI and *Xba* I digests from a second infant with AMMOL (Fig 5). These results confirm that the *AF1q* gene is in the proximity of the breakpoint cluster in two patients with t(1;11)(q21;q23).

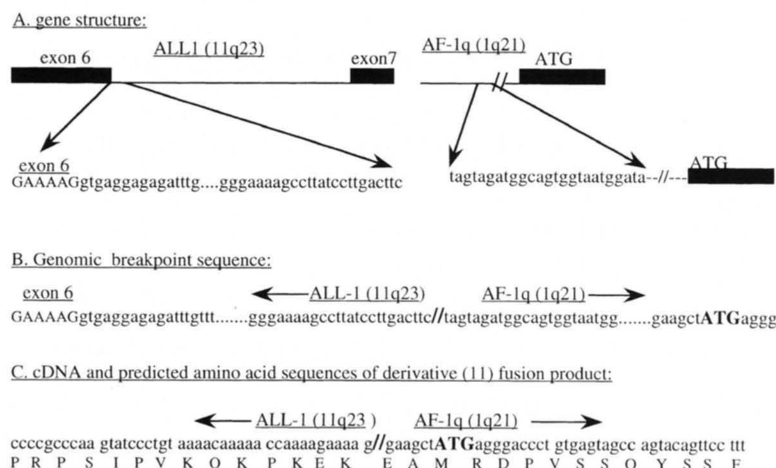

Fig 6. Diagram of the genomic and cDNA fusion products in t(1;11)(q21;q23). (A) Gene structure of the breakpoint region of *MLL* and *AF1q*. (B) Genomic sequence of the breakpoint region of der(11) of patient S. (C) cDNA fusion product of der(11) of patient S.

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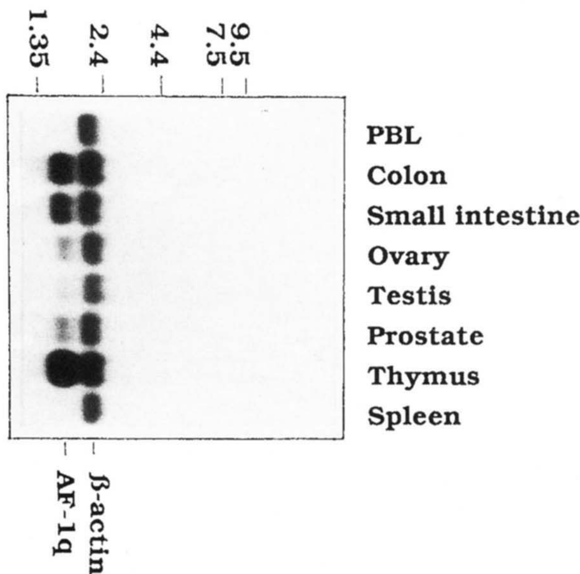

Fig 7. Northern blot analysis of AF-1q mRNA expression in various tissues. Hybridization with AF1q cDNA shows 1.8kb mRNA (lower band). Upper band was hybridized with β-actin probe.

The t(1;11)(q21;q23) breakpoint creates a fusion cDNA product of MLL/AF1q *genes in an AMMOL patient.* Previous molecular analysis of six different chromosomal partners translocated to 11q23 always resulted in the formation of a fusion cDNA.[9,10,15,17-19] To examine whether this is also the case here, we have used RT-PCR to determine the sequence of the MLL/AF1q fusion cDNA in the leukemic patient S with t(1;11)(q21;q23). The results, depicted in Fig 6, show that the translocation in der(11) creates a fusion cDNA between MLL and AF1q in this patient. The translocation breakpoint in chromosome 1 of patient S is particularly informative because it occurs six nucleotides upstream of the first ATG, thus creating a predicted fusion protein of the MLL product with the entire AF1q cDNA in der(11), but in the reciprocal der(1) the open reading frame is destroyed and thus does not give rise to a protein product.

Expression of the AF1q mRNA. To examine the tissue distribution of AF1q mRNA, we have performed Northern blot analysis of poly(A)-selected mRNA from different tissues (Fig 7). A single mRNA species of 1.8 kb was detected, consistent with the longest cDNA clone isolated. AF1q mRNA is expressed in various tissues and it can be readily detected in the colon and small intestine. Surprisingly, its expression in hematopoietic tissues is quite restricted. AF1q mRNA is abundant in the thymus, but is undetectable in peripheral hematopoietic tissues such as peripheral blood and spleen, suggesting that its expression is highly regulated during lymphocyte differentiation.

In light of the specific expression pattern in hematopoietic tissues, it was of interest to determine the expression of the AF1q mRNA in leukemic cells. We used RT-PCR to examine the relative expression of AF1q mRNA in leukemic cells of various lineages (Fig 8). Despite its restricted expression in normal hematopoiesis to immature T cells, AF1q mRNA is expressed in all leukemic cells examined. Irrespective of their differentiation status, both lymphoid (B, pre-B, and T cells) and myeloid (in the monocytic cell line U937; data not shown) leukemic cells expressed AF1q mRNA at levels comparable with those found in thymocytes.

DISCUSSION

Chromosomal translocations involving band 11q23 have been described in various hematopoietic malignancies, including acute leukemias (monocytic, myelomonocytic, lymphoblastic, mixed, or undifferentiated).[5] One of the most striking features of 11q23 translocations is the multitude of chromosomal partners (at least 15) involved in reciprocal translocations with the *MLL* locus.[5] Typically, each of these translocations results in a specific leukemic phenotype; for instance, patients with t(4;11) characteristically present with biphenotypic ALL/AML whereas patients with t(9;11), t(6;11), and t(11;17) present with AML.[6,7]

To date, six of the translocation partner genes of the 11q23 have been cloned. These include 4q21, 9p21-22, 19p13, 1p32, 6q27, and 17q25.[9,10,15,17-19] In each of these cases the creation of two reciprocal fusion proteins is predicted. The results of these molecular analyses suggest that interruption of the structure of the putative transcription factor *MLL* and the formation of fusion proteins alters its specificity and contributes to the uncontrolled growth that results in leukemia. However, several questions are still open and some of these are: (1) Do the fusion partners of 11q23 contribute any structural components to *MLL* or only provide promoters, initiation, or termination codons? (2) What are the common structural requirements contributed by the fusion partner? (3) Which of the two chromosomal derivatives [der(11), or der(4;6;9;17;19;1p32)] is responsible for oncogenesis? (4) What determines the particular leukemic phenotype associated with specific fusion partners?

In an attempt to clarify some of these questions, we cloned the t(1;11)(q21;q23) translocations from a patient with AMMOL. The involvement of the *MLL* gene was shown at the molecular level in leukemic cells from this patient. The genomic breakpoint from leukemic cells of a patient with t(1;11)(q21;q23) was cloned using a probe within the breakpoint cluster of the *MLL* gene. Using a repeat-free chromosome 1 probe, the AF1q cDNA involved in the t(1;11)(q21;q23) was cloned. Chimeric MLL cDNA was isolated by PCR from one of these patients and was shown to contain chromosome band 1q21-derived sequences. Sequence analyses of the genomic breakpoints and of the fusion cDNA products predicted the creation of an in-frame fusion protein in derivative 11, but not in der(1) in leukemic cells from this patient. Sequence analysis of the AF1q cDNA predicted a protein of 90 amino acids. The AF1q mRNA is expressed in a number of tissues, including high levels in the thymus, whereas none of the mature hematopoietic cells at the periphery express AF1q mRNA. In contrast, AF1q mRNA is expressed in all hematopoietic tumors tested regardless of their degree of differentiation.

The molecular analysis of the t(1;11)(q21;q23) helps us clarify some of the questions regarding the oncogenic mechanisms of the translocations involving the *MLL* gene. We

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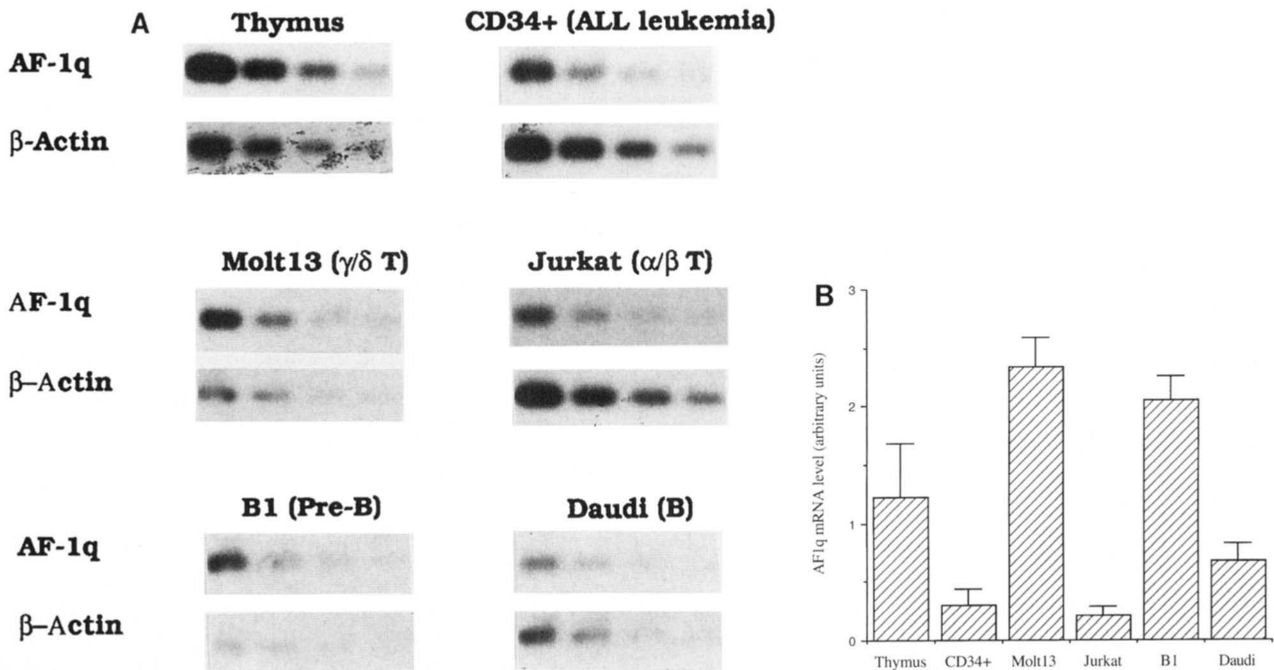

Fig 8. RT-PCR analysis of the expression of AF-1q mRNA in leukemic cells. (A) Five, 2.5, 1.25, and 0.625 ng of total RNA were used (in respective lanes from left to right) for freshly isolated thymocytes (thymus), CD34$^+$ (AML) leukemic cells, and Jurkat (leukemic T-cell line). Tenfold lower total RNA was used for amplification of cDNAs from B1 (ALL leukemic cell line) and Molt13 cell ($\gamma\delta$ leukemic cell line) and five times lower concentration of cDNA was used for Duadi cells. The PCR products were then run in 1.5% agarose gel, transferred to nylon membrane, and hybridized with 5′-labeled cDNA-specific deoxyoligonucleotides, (for AF1q PCR: TTCTTAGGAGGGGCTGCATTGCAG and for β-actin: CTGGCATCGTGATGGACTCCGGTGAC). (B) Relative levels of AF1q mRNA calculated from densitometery of three individual RT-PCR experiments and normalized according to β-actin expression.

describe here molecular evidence that suggests which one of the two fusion *MLL* derivatives is the oncogenic product. This evidence comes from analysis of the t(1;11)(q21;q23) in an AMMOL patient that predicts the formation of only one fusion protein, that of der(11). The reciprocal derivative in this translocation not only lacks a fusion product, but also results in loss of the open reading frame of the carboxy terminal part of the translocated *MLL* gene. These data suggest that the N-terminal part of the *MLL* gene containing the putative "AT hooks" DNA binding motifs and the DNA methyltransferase motif is the oncogenic part of the molecule. This prediction is consistent with previous evidence based on cytogenetic data.[11,26]

The prediction that the N-terminal portion of *MLL* is the oncogenic protein also suggests that the fused *MLL* uses its own promoter for expression in different types of leukemia. The differences in the phenotypic characteristics observed among leukemias with different fusion partners is thus likely caused by structural differences contributed by the fusion partners rather than differential expression caused by promoter elements.

The cDNA of AF1q encodes a small protein of predicted size of 90 amino acid as confirmed by in vitro translation. The predicted AF1q protein encoded by the cDNA has several interesting features. Such small peptide sequences are often found in various cytokines and growth factors; for instance, the recently cloned 89 amino acid pre–B-cell growth-stimulating factor is encoded by an 1.8-kb mRNA.[27] In addition to its unusually small size, AF1q has other features commonly found in cytokines: the presence of multiple mRNA destabilizing consensus sequences present in its untranslated 3′ end[25] and the restricted and highly regulated expression of its mRNA in normal tissues but abnormal expression in tumor cells.

ACKNOWLEDGMENT

We thank Drs E. Canaani and Y. Gu for the generous supply of *MLL* probes and for their continued advice; D.F. Bu for technical advice; and Dr M. Freedman, T. Grunberger, and W. Vanek for the provision of patient tissues and clinical information, and I. Dube for cytogenetic analysis.

REFERENCES

1. Croce CM: Role of chromosome translocations in human neoplasia. Cell 49:155, 1987

2. Gauwerky CE, Croce CM: Chromosomal translocations in leukaemia. Semin Cancer Biol 4:333, 1993

3. Kearney L, Bower M, Gibbons B, Das S, Chaplin T, Nacheva E, Chessells JM, Reeves B, Riley JH, Lister TA, et al: Chromosome 11q23 translocations in both infant and adult acute leukemias are detected by in situ hybridization with a yeast artificial chromosome. Blood 80:1659, 1992

4. Petkovic I, Konja J, Nakic M: Cytogenetic analysis in children with acute nonlymphocytic leukemia. Cancer Genet Cytogenet 58:155, 1992

5. Young BD: Cytogenetic and molecular analysis of chromosome 11q23 abnormalities in leukaemia. Baillieres Clin Haematol 5:881, 1992

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6. Pui Ching-Hon CWM, Look AT: Biology and clinical significance of cytogenetic abnormalities in childhood acute lymphoblastic leukemia. Blood 76:1449, 1990

7. Heim SMF: Cancer Cytogenet 1987

8. Djabali M, Selleri L, Parry P, Bower M, Young BD, Evans GA: A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukaemias. Nat Genet 2:113, 1992

9. Gu Y, Nakamura T, Alder H, Prasad R, Canaani O, Cimino G, Croce CM, Canaani E: The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to *Drosophila trithorax,* to the AF-4 gene. Cell 71:701, 1992

10. Tkachuk DC, Kohler S, Cleary ML: Involvement of a homolog of *Drosophila trithorax* by 11q23 chromosomal translocations in acute leukemias. Cell 71:691, 1992

11. Harris BN, Davis EM, LeBaeau MM, Bitter MA, Kaminer LS, Morgan E, Rowley JD: Variant translocations (;11): Identification of the critical genetic rearrengement. Cancer Genet 30:171, 1988

12. Ma Q, Alder H, Nelson KK, Chatterjee D, Gu Y, Nakamura T, Canaani E, Croce CM, Siracusa LD, Buchberg AM: Analysis of the murine All-1 gene reveals conserved domains with human ALL-1 and identifies a motif shared with DNA methyltransferases. Proc Natl Acad Sci USA 90:6350, 1993

13. Gu Y, Alder H, Nakamura T, Schichman SA, Prasad R, Canaani O, Saito H, Croce CM, Canaani E: Sequence analysis of the breakpoint cluster region in the ALL-1 gene involved in acute leukemia. Cancer Res 54:2326, 1994

14. Hilden JM, Chen CS, Moore R, Frestedt J, Kersey JH: Heterogeneity in MLL/AF-4 fusion messenger RNA detected by the polymerase chain reaction in t(4;11) acute leukemia. Cancer Res 53:3853, 1993

15. Nakamura T, Alder H, Gu Y, Prasad R, Canaani O, Kamada N, Gale RP, Lange B, Crist WM, Nowell PC, Croce CM, Cananni E: Genes on chromosomes 4, 9, and 19 involved in 11q23 abnormalities in acute leukemia share sequence homology and/or common motifs. Proc Natl Acad Sci USA 90:4631, 1993

16. Thirman MJ, Gill HJ, Burnett RC, Mbangkollo D, McCabe NR, Kobayashi H, Poel ZVS, Kaneko Y, Morgan R, Sandberg AA, Chaganti RSK, Larson RA, Le Beau MM, Diaz MD, Rowley JD: Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations [see comments]. N Engl J Med 329:909, 1993

17. Domer P, Fakharzadeh SS Chen C-S, Jockel J, Johanson L, Silverman GA, Kersy JH, Korsmeyer SJ: Acute mixed-lineage leukemia t(4;11)(q21;q23) generates an MLL-AF4 fusion product. Proc Natl Acad Sci USA 90:7884, 1993

18. Bernard OA, Mauchauffe M, Mecucci C, Berghe HVD, Berger R: A novel gene, AF-1p, fused to HRX in t(1;11)(p32;q23), is not related to AF-4, AF-9 nor ENL. Oncogene 9:1039, 1994

19. Prasad R, Leshkowitz D, Gu Y, Alder H, Nakamura T, Saito H, Hubner K, Berger R, Croce CM, Canaani E: Leucine-zipper dimerization motif encoded by the AF17 gene fused to ALL-1 (MLL) in acute leukemia. Proc Natl Acad Sci USA 91:8107, 1994

20. Schichman SA, Caigiuri MA, Gu Y, Strout MP, Canaani E, Bloomfield CD, Croce CM: All-1 partial duplication in acute leukemia. Proc Natl Acad Sci USA 91:6236, 1994

21. Cohen A, Grunberger T, Vanek W, Dube ID, Doherty PJ, Letarte M, Roifman C, Freedman MH: Constitutive expression and role in growth regulation of interleukin-1 and multiple cytokine receptors in a biphenotypic leukemic cell line. Blood 78:94, 1991

22. Sambrook J, Fritsch EF, Maniatis, T: Molecular Cloning: A Laboratory Manual (ed 2). Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 1989

23. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol chloroform extraction. Anal Biochem 162:156, 1987

24. McCabe NR, Burnett RC, Gill HJ, Thirman MJ, Mbangkollo D, Kipiniak M, Nelle EV, Poel ZVS, Rowley JD, Diaz MO: Cloning of cDNAs of the MLL gene that detect DNA rearrangements and altered RNA transcripts in human leukemic cells with 11q23 translocations. Proc Natl Acad Sci USA 89:11794, 1992

25. Wilson T, Treisman R: Removal of poly(A) and subsequent degradation of c-fos mRNA facilitated by 3′ AU-rich sequences. Nature 336:396, 1988

26. Raimondi SC, Peiper SC, Kitchingman GR, Behm FG, Williams DL, Hancock ML, Mirro JJ: Childhood acute lymphoblastic leukemia with chromosomal breakpoints at 11q23. Blood 73:1627, 1989

27. Nagasawa T, Kikutamni H, Kishimoto T: Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc Natl Acad Sci USA 91:2305, 1994

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