[CANCER RESEARCH 53, 3800–3803, August 15, 1993]

Southern Blot Analysis of *ALL-1* Rearrangements at Chromosome 11q23 in Acute Leukemia

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ABSTRACT

The chromosome 11q23 band is a genetic region frequently involved in nonrandom karyotypic abnormalities of acute leukemia. A genomic locus named *ALL-1* or *MLL*, where 11q23 breakpoints are clustered, has been recently cloned and characterized. We have made use of an *ALL-1*-specific probe in Southern blot experiments to analyze the configuration of this gene in a large series of acute leukemia patients, representative of all different myeloid and lymphoid subtypes.

Nine of 145 cases (6.2%) showed abnormal *ALL-1* restriction fragments in leukemic DNAs. Of these nine cases, five patients in whom karyotypic data were available displayed chromosome 11q23 aberrations, including t(4;11) (three cases) and t(9;11) (two cases). Immunophenotypic and morphocytochemical characterization of *ALL-1*-rearranged acute leukemia revealed prevalence of poorly differentiated B lymphoid and/or monoblastic features. Considering the whole series, *ALL-1* rearrangements were significantly associated with female sex, higher white blood cell counts at presentation, and very poor clinical outcome. The presence of residual disease was molecularly documented in one case at the time of clinical remission after induction treatment and was followed by early relapse. We conclude that *ALL-1* rearrangements are new molecular markers of human leukemia with considerable diagnostic and prognostic relevance.

INTRODUCTION

The chromosome 11q23 region is involved in a number of karyotypic aberrations described in AL,[2] including t(4;11) (q21;q23), t(9;11) (p22;q23), t(6;11) (q27;q23), t(1;11) (p32;q23), del 11 (q23), and others (1–9). Clinical characteristics of AL patients with 11q23 cytogenetic abnormalities include young age (they are frequently infants less than 1 yr old), massive tumor cell burden, extramedullary involvement, and poor prognostic outcome (1–10). Concerning blast cell features, these tumors may display variable phenotypes, most frequently revealing early B (CD19+/CD10–) ontogeny. However, monoblastic or biphenotypic markers are also observed (4, 8, 9). This heterogeneity probably reflects the involvement of a multipotent progenitor cell able to differentiate into both lymphoid and myeloid lineages.

We and others have recently cloned a genetic locus, named *ALL-1* or *MLL*, where chromosome 11q23 rearrangements are clustered (11, 12). A DNA probe exploring a 14-kilobase region of this locus has been shown to detect specific rearrangements in the majority of AL patients with 11q23 cytogenetic abnormalities (11, 13). Our molecular investigation was now extended to a large series of AL cases with the aim of verifying the incidence and biological and clinical correlations of *ALL-1* rearrangements in these patients. We report here that this alteration is found in 6.2% of ALs and is significantly associated with female sex, hyperleukocytosis, and resistant disease.

Received 3/11/93; accepted 6/5/93.

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[2] The abbreviation used is: AL, acute leukemia.

MATERIALS AND METHODS

Patients and Samples. Leukemic DNAs were obtained from bone marrow or peripheral blood samples of 145 patients (81 males and 64 females) with acute leukemia observed during the period from January 1987 to January 1992 at the Institute of Hematology of the University "La Sapienza" of Rome. The median age was 28.7 yr (range, 1 to 74.6 yr). The diagnosis of AL was done according to standard FAB criteria (14). Immunophenotypic characterization was performed by indirect immunofluorescence using a wide panel of different lineage-associated markers, as already reported elsewhere (15).

DNA Analysis. High-molecular-weight DNA was recovered by digestion with proteinase K, extraction with phenol/chloroform, and precipitation with ethanol. After digestion with the appropriate restriction endonuclease, DNA was electrophoresed in a 0.8% agarose gel, denatured, and transferred onto nitrocellulose membranes. Filters were hybridized overnight with the ^{32}P random priming labeled probe FA4, washed, and exposed 48 to 72 h at –80°C for autoradiography, using intensifying screens. Hybridization and washing conditions have been described into detail elsewhere (16). The FA4 probe is a 480-base pair genomic *Alu*-free insert representative of the *ALL-1* locus and was derived from the previously reported *Dde* I fragment (11, 13). Fig. 1 illustrates the partial restriction map of the *ALL-1* locus with location of the FA4 probe.

RESULTS

Southern blot analysis with multiple enzyme digestions allowed us to explore a region of 14 kilobases inside the *ALL-1* locus. At least two restriction endonucleases (*Eco*RV, *Bam*HI) were used in all cases. Nine of the 145 patients (6.2%) showed abnormal *ALL-1* fragments in their leukemic DNAs. In 5 of these cases, the rearrangements could be detected with both *Eco*RV and *Bam*HI, whereas in the other 4 cases the abnormal bands were identified with *Bam*HI and *Xba*I but not with *Eco*RV. Therefore, two breakpoint clusters were identified, one of 5.4 kilobases falling inside the *Eco*RV fragment and one of 3.2 kilobases, encompassed by the telomeric *Bam*HI and *Eco*RV sites (see Fig. 1). Neither clinical nor biological differences were found in the comparison of these two patient subgroups. In summary, all breakpoints clustered in the 8.4-kilobase *ALL-1* region encompassed by the telomeric *Bam*HI site and the centromeric *Eco*RV site shown in Fig. 1. Some representative experiments are illustrated in Fig. 2.

The correlations between *ALL-1* configuration and biological and clinical features in the whole series are reported in Table 1. The presence of rearrangements was significantly associated with female sex ($P = 0.01$) and with higher median WBC counts at diagnosis ($P < 0.05$). The biological and clinical characteristics of the 9 patients with *ALL-1* rearrangements are shown in Table 2. Two infant leukemias (age, <1 yr) included in this study were both found rearranged at the *ALL-1* locus. Abnormal *ALL-1* restriction fragments were also detected in a 12-yr-old pediatric patient and in 6 adults. According to FAB criteria, 3 of these cases were diagnosed as poorly differentiated (M_{5a}) monoblastic AL; one was classified as M_0 (minimally differentiated myeloid AL), and 5 showed lymphoid L_1 or L_2 morphocytochemical features. Following immunophenotyping, assignment to the myeloid lineage was confirmed in 2 of the 3 M_{5a} cases (Nos. 2 and 5; Table 2) while the third one (No. 1) showed early B (CD19+/

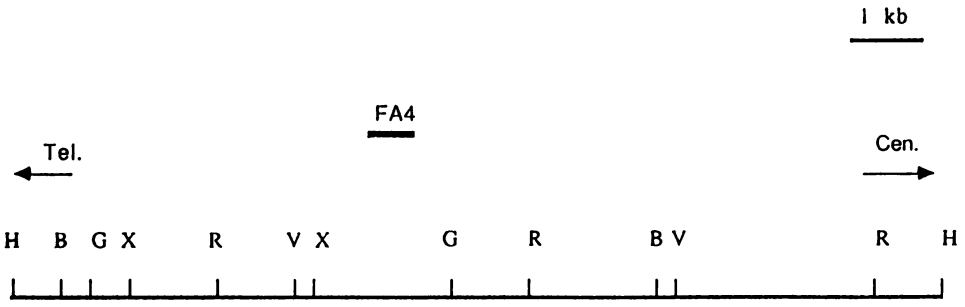

Fig. 1. Partial restriction map of the *ALL-1* locus at chromosome 11q23, with location of the FA4 probe used for hybridization experiments. *H*, *Hind*III; *B*, *Bam*HI; *G*, *Bgl*II; *X*, *Xba*I; *R*, *Eco*RI; *V*, *Eco*RV. *kb*, kilobases; *Tel.*, telomere; *Cen.*, centromere.

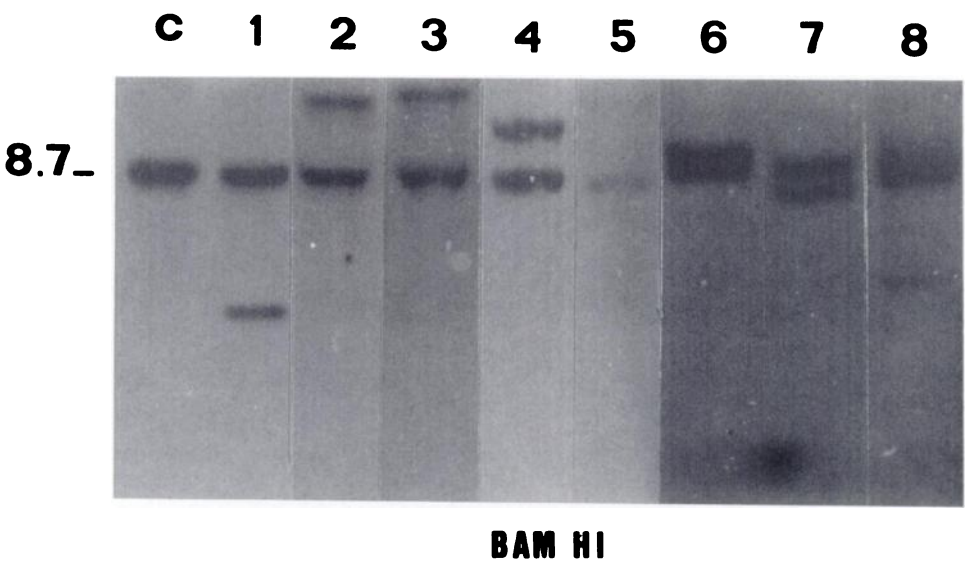

Fig. 2. Southern blot analysis of the *ALL-1* locus in some representative cases. Following digestion of DNAs with *Bam*HI and hybridization with the FA4 probe, additional restriction fragments are visible in 8 leukemic patients (*Lanes 1* to *8*) in addition to the germline 8.7-kilobase band. *C*, control placental DNA.

Table 1 *ALL-1 locus configuration and correlations with clinical and biological characteristics of patients*

	Rearranged (n = 9)	Germline (n = 136)	P value
Age (yr)			
<1	2	0	
1–20	2	62	
>20	5	74	
Sex			
Male	1	80	
Female	8	56	0.01
WBC (10^9/liter)			
Mean	228	39	<0.05
Range	1.9–740	0.3–375	
FAB			
M_0	1	1	
M_1–M_4	0	27	
M_5	3	30	
M_6–M_7	0	5	
L_1	3	43	
L_2	2	24	
L_3	0	3	
Unclassified	0	3	
Immunophenotype			
Myeloid	2	47	
B-lymphoid	4	55	
T-lymphoid	0	27	
Undifferentiated	3	3	
Not available		4	

CD10–) lymphoid markers. Three other cases were classified as early B by immunophenotype (Nos. 3, 6, and 7). Finally, surface marker analysis showed undifferentiated features and did not allow a precise lineage affiliation in the 3 remaining cases (Nos. 4, 8, and 9). In 5 of the 9 patients with *ALL-1* rearrangements, karyotypic examination was available and showed a t(4;11) (q21;q3) in three cases and a t(9;11) (p22;q3) in two.

The response to therapy and clinical outcome was evaluable in 8 cases (patient 8 refused treatment). Following conventional aggressive induction therapy (17–19), five cases showed resistant disease and three achieved complete remission. Of these 3, one relapsed at 5 mo and 2 are in complete remission at 7 and 15 mo, respectively (Table 2). Given the heterogeneous treatment regimens, a statistical comparison of clinical outcome and prognosis between the *ALL-1* rearranged/*ALL-1* germline groups was not feasible.

A Southern blot study of residual disease was performed in 2 cases by analyzing the bone marrow DNAs collected at the time of morphological remission (Fig. 3). In one case, the persistence of a thin abnormal *ALL-1* fragment of the same size than that seen at diagnosis was observed. This patient relapsed 3 mo later. No evidence of residual disease was found in the other case.

DISCUSSION

Recent cloning and characterization of the *ALL-1*/*MLL* gene involved in 11q23 breakpoints have provided new insights into our knowledge of the genetic mechanisms underlying leukemogenesis (11–13, 20–22). The analysis of *ALL-1* complementary DNA indicates that this gene encodes a high-molecular-weight protein with sequence homology to the *Drosophila* trithorax gene (20–22). This latter is known to be active in crucial steps of fruit fly development. Such similarity suggests that *ALL-1* may be a transcription factor involved in the regulation of other genes controlling human development and/or differentiation. In addition, both chromosome 4 and chromosome 11 breakpoints in the t(4;11) cluster in a small DNA region of 8 to 9 kilobases, and heptamer- and nonamer-like sequences have been found in the proximity of breakpoints, indicating the possible involve-

Table 2 *Clinical and biological features of ALL-1-rearranged patients*

Patient	Age/sex	FAB	WBC ($\times 10^9$/liter)	Immunophenotype	Karyotype	Treatment outcome
1	12/F	M_{5a}	340	Early B[a]	t(9;11) (p22;q23)	Resistant/lost to follow up
2	1/M	M_{5a}	40	Myeloid[b]	t(9;11) (p22;q23)	Resistant/dead
3	28/F	L_1	380	Early B	t(4;11) (q21;q23)	CR[c] + 15 mo
4	1/F	L_1	12	Undifferentiated[d]	NA	Resistant/dead
5	63/F	M_{5a}	1.9	Myeloid	NA	Lost to follow up
6	35/F	L_2	740	Early B	t(4;11) (q21;q23)	CR/relapsed at 5 mo/dead
7	64/F	L_2	194	Early B	t(4;11) (q21;q23)	Resistant/dead
8	52/F	L_1	328	Undifferentiated[e]	NA	CR + 7 mo
9	19/F	M_0	23	Undifferentiated[f]	NA	Resistant/dead

[a] TdT+/CD19+/CD10−.
[b] CD13+/CD33+.
[c] CR, complete remission; NA, not available.
[d] TdT−/DR+/CD19+.
[e] TdT+/DR+/CD19−.
[f] TdT−/DR−/CD19−/Cd13−/CD33+/CD7+.

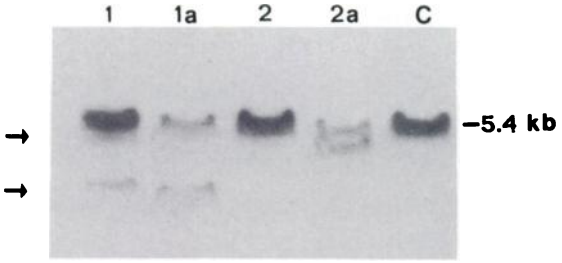

Fig. 3. Southern blot hybridization of diagnostic (*Lanes 1a* and *2a*) and remission (*Lanes 1* and *2*) bone marrow DNA in two patients. The specific *ALL-1* rearrangement detected at presentation was not further visible at remission in one case (*Lanes 2a* and *2*; Patient 3 in Table 2) and persisted as a thin residual band in the other case (*Lanes 1a* and *1*; Patient 6 in Table 2). *C*, control placental DNA. The 5.4-kilobase germline band detectable on *Eco*RV digests is indicated by the *bar*. Rearranged bands are indicated by the *arrows*.

ment in this recombination of the V-D-J recombinase active in immunoglobulin gene rearrangements (23).

Besides its biological significance, *ALL-1* is considered to be a novel tumor-specific marker with relevant diagnostic and prognostic usefulness, particularly for characterization studies of leukemia at diagnosis and for the monitoring of residual disease.

In the present analysis, we confirm the phenotypic and clinical correlations previously observed in AL patients with karyotypic 11q23 aberrations. Our study showed the consistent association of *ALL-1* rearrangement with infant leukemias (24), hyperleukocytosis, and very poor clinical outcome. While these data were in line with those available from karyotypic studies, the 8:1 female:male ratio of *ALL-1* rearrangements observed here was considerably higher than that previously reported (1, 2, 4).

As to leukemic cell ontogeny, we found a prevalence of early B lymphoid, undifferentiated, or immature monoblastic markers. Together with the extremely poor clinical outcome of *ALL-1*-rearranged patients, these data contribute to classify this disease as a multipotent stem cell tumor in which conventional chemotherapy regimens are usually ineffective in remission induction. Finally, we have shown the use of this new leukemic marker to specifically identify residual disease at remission after induction treatment. However, since the sensitivity of Southern analysis is limited, polymerase chain reaction strategies should be developed in the future in order to better evaluate the quality of remission.

In conclusion, we believe that, based on both the molecular abnormality and the stem cell phenotypic features of this leukemic subset, individually tailored highly aggressive treatments should be considered for these patients, including the use of allogeneic bone marrow transplantation as consolidation therapy in the first complete remission.

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