Detection of Minimal Residual Disease in Leukemic Patients with the t(10;14)(q24;q11) Chromosomal Translocation

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ABSTRACT

Early relapse and minimal residual disease during clinical remission was examined in two patients having acute T-cell leukemia/lymphoma with the t(10;14)(q24;q11) chromosomal translocation. Molecular probes which can detect T-cell receptor α/δ clonal rearrangements and a TCL-3 probe which can detect the clonal rearrangement due to the chromosomal translocation failed to detect the leukemic clones during clinical remission by Southern filter hybridization. However, application of the polymerase chain reaction technology in amplification of the t(10;14)(q24;q11) chromosomal juncture during clinical remission permitted us to increase the detection level of neoplastic cells up to 1 leukemic cell/125,000 normal cells using 1 μg of DNA. Amplified junction fragments were detected in both patients. In one case, during the period of clinical remission no amplified fragments were detected.

INTRODUCTION

Similarly to other hematopoietic malignancies the majority of lymphoid neoplasms are of monoclonal origin (1). Previously, the determination of monoclonality within lymphoid neoplasms has been largely restricted to B-cell neoplasms which display either one of the immunoglobulin light chain isotypes (κ or λ), a specific cytogenetic abnormality, or two different polymorphic forms of glucose-6-phosphate dehydrogenase isozyme in the patient (2-4).

Recently the development of a genetic approach, based upon molecular analysis of immunoglobulin and T-cell receptor genes rearrangement, has been used to determine monoclonality and lineage specificity in tumors without lineage-specific surface determinants (5-13). In addition, molecular probes have been used in the detection of residual or recurrent disease not detected by routine diagnostic methods (14). The sensitivity of Southern filter analysis is useful in the detection of a clonal cell population only if it makes up 1-5% of the total cell population. Attempts to amplify the immunoglobulin or T-cell receptor rearrangements are limited due to the large variety of alternative Variable (V), Diversity (D), and Joining (J) segments which might be also present in the normal cells in addition to the neoplastic cells.

On the other hand, cytogenetic abnormalities, such as translocations, inversions, and deletions, are nonrandomly associated with specific types of human leukemias and lymphomas (3, 15). In these cases detection of patient genomic DNA rearrangements due to the chromosomal abnormality is a unique tumor-specific marker. Recently we have defined a novel gene rearrangement in acute T-cell leukemia/lymphoma with a t(10;14)(q24;q11) chromosomal translocation (16, 17). These translocation breakpoints occur within the T-cell receptor δ-chain locus on the Dδ2 segment on chromosome 14q11 and are clustered within a 263 base pair region of the TCL-3 locus on chromosome 10q24. (17)

In the present study we have applied the polymerase chain reaction (18) to amplify the t(10;14)(q24;q11) chromosomal juncture for the detection of minimal residual disease during remission.

MATERIALS AND METHODS

Patient Material

Peripheral blood lymphocyte samples were collected during the course of the disease from two patients, DW and JM, with T-cell lymphoblastic leukemia/lymphoma and acute T-cell leukemia, respectively. Both patient’s leukemic cells carry the t(10;14)(q24;q11) chromosomal translocation (16, 17).

Samples. DW and JM are blood samples collected before treatment, during the course of disease. DW-R1, DW-R2, DW-R3, and JM-R blood samples were collected monthly during clinical remission.

Lymphocytes were isolated from heparinized blood by Ficoll-Hypaque separation (Pharmacia). Other cell lines included in the present study were: 639 AD4, a somatic cell hybrid derived from the leukemic T-cells of DW which has retained the chromosome 10q+ (16); 648 BD4, a somatic cell hybrid derived from the leukemic T-cells of JM which has retained all the relevant chromosomes (10, 10q+, 14, 14q- (17); and 648E 10 (JM), a somatic cell hybrid derived from the leukemic T-cells of JM which has retained the chromosome 10q+. (17).

Southern Blot Analysis

Ten μg of high molecular weight DNA from patients or human placental DNA and 20 μg of high molecular weight 648 BD4 (JM) somatic cell hybrid DNA were digested to completion by various restriction enzymes, fractionated on a 0.6% agarose gel (Sigma), and transferred to either a nitrocellulose or a PVDF Immobiline membrane (Millipore) as previously described by Southern (19). Hybridization conditions of the nick-translated DNA probes to a specific activity of 5 × 10^6 cpm/μg with the DNA-bound filters were previously described (16).

DNA Probes

(a) PSKW0.6H is a 0.6H fragment located 27 kilobases 5' to TCR3 Ca segment; (b) PJK J6 2.4 E/B (17); (c) PJK J6 3.0 SacI (17); (d) P1011J6, 5.OE (17); (e) PJK 1.7 E/B, a TCL-3 probe (17).

Amplification of the t(10;14)(q24;q11) Chromosome Juncture

Amplification with Tag DNA polymerase was performed according to the manufacturer’s instructions (Perkin Elmer Cetus, Norwalk, CT). Target sequences were amplified from 1 μg of genomic DNA in a 100-μl 1× PCR buffer (10 mM Tris HCl, pH 8.3-50 mM KCl-1.5 mM MgCl2-0.01% (w/v) gelatin-2 mM dNTP-1 μM of each of the 2 amplifiers) with 2.5 μl of Tag DNA polymerase. The reaction mixtures were overlaid with 100 μl of paraffin oil and the reaction-capped tubes were incubated for 10 min at 94°C followed by serial cycles of denaturing (1 min at 94°C), annealing (1 min at 65°C), and primer extensions (2 min at 72°C). After the final cycle the tubes were incubated for 10 min at the extension temperature (72°C). The PCR products were extracted

Received 3/20/90; revised 5/21/90.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by CA 25875, CA 39860, American Cancer Society CH 378A, and W. W. Smith Charitable Trust.

2 The abbreviations used are: TCR, T-cell receptor; PCR, polymerase chain reaction.

3 L. Finger, unpublished data.
Fig. 1. Clonal rearrangements at the TCR α/β locus. A, genomic organization of the TCR α/β locus. Top line: boxes, structural genes; closed and hatched boxes, coding exons and the 3'-untranslated region, respectively. Restriction sites: B, BamHI; E, EcoRI; H, HindIII. PKSW 0.6H, PKJKJ2 E/B 2.4, PKJKJ 3.0 SacI, and P101JJE 5.0, the locations of the probes used to detect rearrangements. B, clonal rearrangement of the TCR Jα locus in the DW DNA samples. Southern blot hybridization of BamHI-digested DNAs probed with the PKSW 0.6H probe. C, clonal rearrangement at TCR Jα locus in JM’s DNA samples. Southern blot hybridization of EcoRI digested DNAs probed with the PKJKJ 2.4 probe. (D) Clonal rearrangement at the TCRJβ locus associated with a t(10;14)(q24;q11) chromosomal translocation. Southern blot hybridization of BamHI-digested DNAs probed with the PKJKJ 3.0 SacI probe. PL, human placental DNA; DW and JM, patient DNA samples carrying the t(10;14)(q24;q11) chromosomal translocation; DW-R, DW-R2, JM-R, DNA samples collected during clinical remission from both patients; 648 BD (JM), a somatic cell hybrid derived from JM’s leukemic T-cells which has retained all of the relevant chromosomes (10, 10q+, 14, 14q-). Germline DNA fragments (GL) and rearranged DNA fragments (R) are indicated. kb, molecular sizes in kilobases.

Oligonucleotides

Oligonucleotides were synthesized and high performance liquid chromatography purified by Research Genetics (Huntsville, AL). Probe A. 17-mer 5'-TCGCGCTGTCATTCACC-3' is a chromosome 10 sequence complementary to the internal sequence in the chromosome 14 TCRβ-probe PJKJ6 3.0 Sad. A 13.9-kilobase BamHI-rearranged fragment and a 3.6-kilobase BamHI germline fragment. The rearranged fragment was detected only in DW’s DNA and not in two remission samples of DW (DW-R, and DW-R2), obtained for evaluation while the patient was in remission. Another probe PJKJ5 2.4 E/B which spans the chromosome 14 Jβ2 segment detects a 5-kilobase EcoRI germline fragment and a 3-kilobase EcoRI-rearranged fragment in the JM somatic cell hybrid 648 BD (Fig. 1, A and B) located on chromosome 14 19-mer 5’-ATCCGTCAAGGAAGCGGCT-3’ is a chromosome 10 sequence complementary to an internal segment of the amplified sequence.

RESULTS

Detection of Clonal Rearrangement. During our search for the t(10;14)(q24;q11) chromosomal translocations in acute T-cell leukemias and lymphomas we have applied a battery of TCR α/β probes which spans the entire TCR α/β locus to detect DNA rearrangements by Southern blotting analysis.

Using a probe PSKW0.6H (Fig. 1, A and B) located on chromosome 14 ~27 kilobases 5’ to Ca, we have detected a 4.1-kilobase BamHI-rearranged fragment and a 3.6-kilobase BamHI germline fragment. The rearranged fragment was detected only in DW’s DNA and not in two remission samples of DW (DW-R, and DW-R2), obtained for evaluation while the patient was in remission. Another probe PJKJ5 2.4 E/B which spans the chromosome 14 Jβ2 segment detects a 5-kilobase EcoRI germline fragment and a 3-kilobase EcoRI-rearranged fragment in the JM somatic cell hybrid 648 BD (Fig. 1, A and B) located on chromosome 14 19-mer 5’-ATCCGTCAAGGAAGCGGCT-3’ is a chromosome 10 sequence complementary to an internal segment of the amplified sequence. kmol, molecular sizes in kilobases.
...translocation breakpoint. PJK 17 E/B probe also detects a 5.7-kilobase germline BamHI fragment from the normal chromosome 10 allele in the patient samples and in the human placental DNA control (Fig. 2). As expected, this probe detects only the 13.9-kilobase BamHI-rearranged fragment in the hybrid 6488E10 (JM) since this hybrid retained the chromosome 10q+ and not a normal chromosome 10 allele (17). The rearranged 13.9-kilobase BamHI-rearranged fragment was not detected in the samples JM-R, DW-R1, and DW-R2 obtained during clinical remission (Fig. 2).

Although the clonal rearrangement at the TCR α/β locus detected in DW's and JM's DNA may serve as individual clonal markers for each patient in monitoring the progression of the disease, only probes which detect a rearranged fragment due to the chromosomal translocation will serve as a tumor-specific disease, only probes which detect a rearranged fragment due to the chromosomal translocation will serve as a tumor-specific marker for patients with t(10;14)(q24;q11) chromosomal translocation.

Amplification of the t(10;14) Chromosomal Juncture. The molecular cloning of the breakpoint sequences revealed that the translocation occurred into the D6 segment of TCRδ on chromosome 14q11 and within a 263-base pair segment of chromosome 10 (17). In order to amplify the translocation breakpoint junctions we have designed one set of amplifiers with a complementary primer to the 5' end of the translocation breakpoint-flanking sequence on chromosome 10q24 (amplimer C, Fig. 3A) and another set of amplifiers with a reverse complementary primer to the 3' end of the translocation breakpoint-flanking sequence on chromosome 14q11 (amplimer E, Fig. 3A).

Amplification across the t(10;14)(q24;q11) chromosomal junction was carried out using amplifiers C and E as a means to monitor the level of the leukemic cells carrying the translocation in the patient's peripheral blood lymphocytes. Amplified fragments were detected in both patients prior to clinical remission. As expected, DW's 486-base pair amplified fragment is larger than JM's 233-base pair amplified fragment because of the additional 263 nucleotides adjacent to DW's breakpoint junction and amplimer G has a reverse complementary primer to the 3' end of the extra 263-base pair sequence on chromosome 10 in DW's breakpoint junction. An internal oligonucleotide hybridization probe, Probe A, identifies amplified fragments between amplimer C and E or C and D, while Probe B identifies only amplified fragments which contain the 263-base pair additional sequence adjacent to the breakpoint in DW's DNA. All the amplimers include an anchor (AN) sequence with endonucleases restriction sites. B, ethidium bromide-stained PCR-amplified DNA fragments separated on a 4% agarose gel.

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As for the second patient DW, PCR amplification across the chromosomal juncture of the samples confirmed the initial Southern hybridization results (Fig. 1B and Fig. 2). A 486-base pair amplified fragment was detected only in the sample DW probed with Probe A (Fig. 3C, Lanes 5–7, and Fig. 4, Lane 1) or with Probe B (Fig. 3D, Lanes 5–7) and not in samples DW-R1 and DW-R2 obtained while the patient reached clinical remission (Fig. 3, Lanes 6 and 7, and Fig. 4, Lanes 2 and 3). Shortly after those examinations patient DW relapsed. A 485-base pair fragment resulting from amplification between amplimers C and D (Fig. 4, Lanes 1 and 4) was detected in peripheral blood sample DW-R1. Controls in the experiment included a JM 233-base pair fragment amplified between amplimers C and E (Fig. 4, Lane 5), a normal chromosome 10 149-base pair fragment amplified between amplimers C and E (Fig. 4, Lane 7), and a human placental DNA negative control, without a t(10;14) chromosomal translocation (Fig. 4, Lane 6). Subsequently, patient DW relapsed and died due to T-cell lymphoblastic leukemia/lymphoma.

**Amplification Sensitivity.** The amplification sensitivity of our amplification assay was estimated in a mixing experiment. Patient DW's DNA containing the t(10;14)(q24;q11) chromosomal translocation was serially diluted with human placental DNA and 1 µg of the mixed DNA was amplified for 30 cycles between amplimers C and E. As demonstrated in Fig. 5, we can easily detect an amplified fragment in the 1 µg DNA mixture which contained only 10 pg of the patient's DNA carrying the t(10;14) chromosomal translocation. These results indicate that our resolution allows the detection of 1 leukemic cell out of 125,000 normal cells. This resolution can be further improved 10-fold by using 10 µg DNA in the amplification process (data not shown).

**DISCUSSION**

Clonal rearrangements of immunoglobulin or T-cell receptor genes have been detected in the vast majority of lymphoproliferative disorders (14). We have detected and cloned the physiological rearrangements of the TCR α/β locus in both patients (Fig. 1). Each of these clonal rearrangements might be used as an individual specific marker in detecting the neoplastic clone. However, recent observations have described a secondary rearrangement within the TCR α/β locus to a preexisting and functional Vα-Jα segment of the TCRα gene in a T-cell line (21). A similar process was also observed in a mouse tumor B-cell line which escaped from antiidotype therapy due to continuous k-light chain rearrangement (22) and in the λ locus of human B-cell line derived from a large cell lymphoma which has been shown to have undergone secondary rearrangements (23). Thus, it appears that a single rearrangement at the immunoglobulin or TCR loci is an insufficient marker for the detection of monoclonality in lymphoproliferative disorders.

On the other hand, DNA rearrangement due to a chromosomal translocation is a tumor-specific marker. We have demonstrated here the detection of the t(10;14) (q24;q11) chromosomal translocation using two different probes: chromosome 14 TCRJβ1 probe (PJK Jβ 3.0 SacI) and chromosome 10 TCL-3 probe (PJK 1.7 E/B). The TCL-3 probe is the probe of choice in analyzing the breakpoint in T-cell leukemia/lymphoma with a t(10;14)(q24;q11) chromosomal translocation since the Jβ1 probe may also detect a physiological rearrangement at the TCRβ locus.
By Southern blot analysis we are limited by the detection level of a clonal population of cells which have to account for 1–5% of the total cell population. In order to improve the detection level of leukemic T-cells with a t(10;14)(q24;q11) chromosomal translocation within the lymphocyte cell population of the patient we have applied the polymerase chain reaction to amplify the chromosomal juncture.

The specificity of our amplification was demonstrated by (a) the inability to amplify t(10;14) chromosomal junction fragments in normal placental DNA serving as the negative control, (b) detection of the expected size fragments which spans the chromosomal translocation (233 base pairs in JM and 486 base pairs in DW), (c) specific detection of the amplified fragments by a chromosome 10 internal probe which flanks the translocation breakpoint, (d) specific detection of DW’s amplified junction fragment by Probe B (which is an oligomer complementary to an internal sequence in the additional 263-base pair chromosome 10 sequence), and (e) an inability to amplify the 263-base pair sequence between amplifiers F and G in the JM somatic cell hybrid 6488E10 (data not presented). The sensitivity of the PCR method for our analysis permitted us to detect a single leukemic cell out of 125,000 cells using 1 µl of template DNA. This resolution should help lead to a better definition of the term “remission” and early relapse using the peripheral blood lymphocytes and bone marrow from patients with the t(10;14) translocation where the chromosomal breakpoint is at the TCL-3 locus.

REFERENCES

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