Sensitive Detection of Clonal Antigen Receptor Gene Rearrangements for the Diagnosis and Monitoring of Lymphoid Neoplasms by a Polymerase Chain Reaction-Mediated Ribonuclease Protection Assay

By Hendrik Veelken, Benjamin Tycko, and Jeffrey Sklar

This report describes a novel assay involving the polymerase chain reaction (PCR) and RNase protection for the rapid and sensitive detection of malignant lymphoid cells by nucleotide sequences within their individual rearranged gamma T-cell receptor (TCRG) genes. In this assay, clonal rearrangements are amplified from the DNA of diagnostic tumor specimens using a consensus V segment primer and a consensus J segment primer to which the promoter for T7 RNA polymerase has been appended. The PCR product from this amplification is transcribed into a radiolabeled RNA probe. Test RNA transcribed from the opposite DNA strand is synthesized by similar methods from TCRG genes of a subsequent biopsy specimen. The test RNA is hybridized with the probe, and mismatched nucleotide sequences in the RNA hybrids are digested by RNase A. Detection of fully protected probe by means of polyacrylamide gel electrophoresis and autoradiography indicates the presence of malignant cells in the test specimen. Dilution experiments with DNA of cell lines from acute lymphoblastic leukemias (ALLs) show that detection of one tumor cell among 10^8 normal bone marrow cells is usually possible. Residual disease was also successfully detected in several cases of ALL during clinical remission, including detection in one case at the 10^-4 level. The procedure described here may provide a simplified and rapid method for the sensitive diagnosis and monitoring of lymphoid malignancies. This procedure should be applicable to most antigen receptor genes, and unlike most comparable methods, requires neither analysis of nucleotide sequence nor synthesis of tumor-specific oligonucleotide probes or primers. © 1991 by The American Society of Hematology.

Diagnosis of hematolymphoid neoplasms usually involves identification of morphologic characteristics in biopsy tissues and examination of antigen markers on malignant cells. In addition, cytogenetic studies for chromosomal abnormalities and Southern blot analysis for DNA rearrangements often provide important genotypic information distinguishing between neoplastic and benign processes. Unfortunately, even the combined application of all these methods generally fails to detect a particular malignant clone if that clone comprises less than 1% of the total cells in the population being examined. In some instances, double-color flow cytometry appears to be capable of detecting cells of the malignant phenotype with a sensitivity as low as 1:10,000.1

Recently, application of the polymerase chain reaction (PCR) has led to procedures capable of detecting malignant cells with very high sensitivity through the amplification of DNA across breakpoints in chromosomal translocations. However, a number of considerations limit the usefulness of this approach. First, different types of translocations occur in specific forms of lymphoid neoplasia. Therefore, detection of malignant cells by this method presupposes knowledge or at least a strong suspicion of the specific form of neoplasm represented by a given case. Second, even in a particular form of lymphoid neoplasia, not all cases will contain a chromosomal translocation. Third, the position of breakpoints in one type of translocation may vary from case to case, complicating or preventing amplification of DNA across the breakpoints. Fourth, although sequences of breakpoints for different translocations are being determined at an increasing rate, many important translocation breakpoints have not yet been analyzed at the molecular level.

An alternative kind of genotypic marker for detection of lymphoid neoplasms is the configuration of the V, D (when present), and J segments within rearranged antigen receptor genes. The markers created by these gene rearrangements, unlike chromosomal translocations, are not specific for neoplasia per se, but can serve as markers for clonal proliferation, a feature characteristic of neoplasia, and for a particular clone of neoplastic cells. V-(D)-J rearrangements have been used for some time to diagnose and monitor lymphoid neoplasia using Southern blot hybridization. More recently, we and others have used specific DNA sequences within rearranged antigen receptor genes as markers for highly sensitive tumor detection by PCR. The critical part of the DNA used for this purpose is the sequence of nucleotides created at the junction of rearranged segments by the combined effect of small deletions of DNA from the ends of the rearranging segments and the insertion of short stretches of random nucleotides between the segments before their joining. Our own approach has relied on the presence of conserved sequences within V and J segments, permitting the amplification of rearrangements from diagnostic tumor samples with oligonucleotide primers containing sequences complementary to these conserved sequences. After determining the sequence of DNA across the V-(D)-J junction, a tumor-specific primer complementary to the junctional sequence is constructed and used, together with a flanking, nonspecific primer, to test for...
tumor cells within a biopsy specimen by PCR of DNA extracted from the biopsy tissue. In this way, we have been able to routinely detect residual tumor cells making up as little as one in $10^5$ total cells within a single assay. Theoretically, this approach is applicable to immunoglobulin heavy chain genes and all T-cell receptor genes; although our efforts so far have focused on the gamma T-cell receptor (TCRG) gene because of the simplicity of this gene and the case in finding conserved sequence primers for the initial amplification reaction.

Major disadvantages of detecting lymphoid neoplasia using PCR primers for junctional sequences of rearranged antigen receptor genes are that the approach requires determination of the junctional region sequence and the construction of primers specific for this sequence in each case. Among the negative ramifications of these requirements are that the full procedure is rather laborious, takes significant time before results can be obtained, and calls for special and costly instrumentation. To avoid these problems, we have devised a modified approach for sensitive detection of lymphoid neoplasia based on analysis of clonotypic junctional sequences in rearranged antigen receptor genes. This procedure uses conserved region primers to generate both probe and target RNA molecules for a given tumor, followed by RNase digestion of hybrids between the probe and target to eliminate any imperfect hybrids. Analyses of two cell lines derived from acute lymphoblastic leukemias (ALLs), as well as fresh ALL, indicate that this modified approach can generally detect lymphoid neoplasia with the same sensitivity as the earlier version, but much more rapidly and without the determination of any specific sequence information or construction of tumor-specific oligonucleotide probes or primers.

MATERIALS AND METHODS

Cell lines and bone marrow samples. SUP-B7, a cell line derived from a case of common ALL (cALL), and SUP-T1, a cell line derived from a case of T-lymphoblastic leukemia (T-ALL) were used as sources for homogeneous malignant cells. Diagnostic bone marrow aspirates from patients B and C showed morphologic and immunophenotypic characteristics of cALL. Bone marrow from patient A showed morphologic and immunophenotypic characteristics of T-ALL. Sequences of rearranged TCRG genes involving the V-y1 subgroup in these tumors have been determined previously (Tycko, unpublished results) and are shown in Table 1. Morphologically normal bone marrow specimens were obtained from bones removed during surgical procedures and from donors for allogeneic bone marrow transplantsations. All bone marrow samples were obtained with the informed consent of the donors.

**Table 1. V-y1-J-y Junctional Structure of TCRG Rearrangements**

<table>
<thead>
<tr>
<th>Case</th>
<th>Δ3 V-y1</th>
<th>N Insertion</th>
<th>Δ5 J-y</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUP-B7 (V-y4)</td>
<td>-9</td>
<td>CTCCCTGGAAG</td>
<td>-1</td>
</tr>
<tr>
<td>SUP-T1 (V-y3)</td>
<td>-6</td>
<td>CGGAC</td>
<td>0</td>
</tr>
<tr>
<td>Pt A (V-y2)</td>
<td>-4</td>
<td>GTTGGG</td>
<td>-7</td>
</tr>
<tr>
<td>Pt B (V-y4)</td>
<td>-9</td>
<td>GGAG</td>
<td>-12</td>
</tr>
<tr>
<td>Pt C (V-y2)</td>
<td>-8</td>
<td>C</td>
<td>-16</td>
</tr>
<tr>
<td>Pt A (V-y8)</td>
<td>-17</td>
<td>AAGGCC</td>
<td>-16</td>
</tr>
</tbody>
</table>

**PCR.** DNA was extracted from cultured cells and bone marrow by standard methods. Rearranged TCRG genes were amplified from 1 to 2 µg of genomic DNA using oligonucleotide primers specific for the conserved regions within the V-y1 and J-y7 gene segments. Primers were constructed with an automated DNA synthesizer (Applied Biosystems, Foster City, CA). The sequences of the primers are given in Table 2. The primers J-y7 and V-y7 carry at their 5' ends the sequence of the promoter for the T7 RNA polymerase. PCR amplifications were performed in an automated thermal cycler (Perkin Elmer Cetus, Norwalk, CT) for 25 cycles with 2.5 U Thermus aquaticus DNA polymerase (Perkin Elmer Cetus) and 50 pmol of each primer, under conditions recommended by the manufacturer. Each amplification cycle consisted of a DNA denaturation step at 94°C for 1 minute (3 minutes in the first cycle), primer annealing at 60°C for 2.5 minutes, and primer extension at 70°C for 1.5 minutes (6 minutes in the last cycle). Using these parameters, each PCR product consisted of a single band of the predicted size when analyzed by agarose gel electrophoresis. Amplifications without genomic DNA were performed alongside each set of PCR reactions to control for possible contamination with spurious templates. These reactions consistently failed to show detectable products in agarose gels.

In vitro transcription. PCR products were treated with 50 µg/mL proteinase K (BRL, Gaithersburg, MD) for 30 minutes at 37°C; extracted with phenol, and precipitated with 70% ethanol. One-twentieth of the PCR product in each reaction was transcribed in vitro with 20 U of T7 RNA polymerase (Stratagene, La Jolla, CA), according to the conditions recommended by the manufacturer. Radiolabeled RNA probes were synthesized in the presence of 2 mm Ci/mL (800 Ci/mmol) α-32P-UTP (Dupont, NEN, Boston, MA), and adenosine triphosphate (ATP), cytosine triphosphate (CTP), and guanosine triphosphate (GTP) at a concentration of 0.4 mmol/L each. Unlabeled test RNA was synthesized with all four ribonucleotides present at a concentration of 0.4 mmol/L. After transcription, the template DNA was digested with 60 U of RNase-free DNase I (Boehringer Mannheim, Germany) for 30 minutes at 37°C. Probes were separated from unincorporated radioisotopic by centrifugation through Sephadex 25 (Pharmacia, Uppsala, Sweden). All transcripts were extracted with phenol and precipitated with 70% ethanol.

**RNase protection assay.** Radiolabeled probes were hybridized with the appropriate test RNA at a ratio of 1:10 in a total volume of 40 µL 85% formamide, 0.4 mol/L NaCl; 1 mmol/L EDTA; and 40 mmol/L piperaidine n-n bis: 2-ethane sulfonic acid (PIPES), pH 5.6. After denaturation for 10 minutes at 95°C, the hybridization mixtures were incubated overnight at 64°C. To digest mismatched nucleotides, 300 µL of 40 µg/mL RNase A (Boehringer Mannheim) in 0.3 mol/L NaCl, 10 mmol/L Tris-HCl (pH 7.5), and 5 mmol/L EDTA was added to each sample, followed by incubation for 30 minutes at an empirically determined temperature of 42 to 50°C. The RNase digestion was terminated by addition of 20 µL of 10% sodium dodecyl sulfate (SDS) and 2 µL 50 µg/mL proteinase K with subsequent incubation for 15 minutes at 37°C. The digested RNA fragments were extracted with phenol, precipitated, and analyzed by electrophoresis through a 6% polyacrylamide gel containing 7.6 mol/L urea for 4 hours at 45 V/cm in TBE buffer (90 mmol/L Tris; 90 mmol/L boric acid; 2 mmol/L EDTA, pH 8.0). For
autoradiographic detection of the protected RNA, XAR-5 film (Kodak, Rochester, NY) was exposed to the gels with an intensifying screen for 8 to 16 hours.

RESULTS

The strategy used for the RNase protection assay to detect lymphoid neoplasia is outlined in Fig 1. DNA is amplified from a diagnostic tumor sample containing a TCRG rearrangement by PCR with primers Vγ1.3 and JγT7. The Vγ1.3 primer anneals to a region of conserved sequence in all nine known Vγ gene segments of the VγI subgroup.15,16 The JγT7 primer anneals to both known functional Jγ segments.15 If the number of N nucleotides equals the number of germline nucleotides deleted during joining of the Vγ and Jγ segments within a TCRG gene rearrangement, the PCR product has a length of 281 bp, including the promoter of the T7 RNA polymerase added at the downstream (3') end of the amplified Jγ segment. In vitro transcription from this template generates a tumor-specific, radiolabeled anti-sense RNA probe 18 nucleotides shorter than the template fragment. DNA from the specimen to be examined for the presence of the tumor-specific TCRG gene rearrangement is amplified by PCR with the VγT7 and JγI7 primers. The VγT7 primer anneals to the same sequence as the Vγ1.3 primer but contains the T7 promoter at its 5' end. The JγI7 primer anneals to the conserved sequence immediately upstream from the JγT7 complementary sequence.15 This PCR product has an average length of 268 bases and is transcribed, beginning from the T7 promoter at the 5' end of the Vγ segment, into a pool of unlabeled, sense test RNA with an average length of 250 nucleotides. After denaturation, the tumor-specific RNA probe is hybridized at high stringency to the test transcript. The resulting RNA-RNA duplexes are then subjected to digestion with RNase A and analyzed by polyacrylamide gel electrophoresis (PAGE) under denaturing conditions.

If the tumor-specific TCRG rearrangement is present in the sample, absence of mismatched bases in the RNA duplex prevents RNase digestion, with the exception of the protruding 5' end of the probe. Presence of the tumor-specific TCRG rearrangement in the sample is thus indicated by a protected radioactive fragment exactly 19 nucleotides shorter than the unhybridized and undigested probe. (The shorter length of the protected fragment relative to the undigested probe permits differentiation between protection of the probe and failure of RNase to digest single-stranded RNA sequences). If the specimen is free of any tumor-specific TCRG gene rearrangement, possible differences in Vγ segments and, more likely, in the N region of rearranged TCRG genes create base mismatches in the...
RNA-RNA hybrids. Unhybridized pyrimidine residues are cleaved by RNase A. Therefore, in specimens lacking tumor-specific rearrangements, only bands smaller than those predicted for perfectly matched probe should be observed.

To test the specificity and sensitivity of strategy outlined in Fig 1, DNA extracted from the cell line SUP-B7 was logarithmically diluted into normal bone marrow DNA. One microgram of DNA from each dilution, from the normal bone marrow used to prepare the dilutions, and from four other normal bone marrow samples was amplified by PCR and transcribed into test RNA, as described above. A radiolabeled RNA probe was synthesized by PCR amplification of 1 µg undiluted SUP-B7 DNA and then by transcription of the PCR product with T7 RNA polymerase. Five percent of this probe was hybridized to one half of each test RNA preparation, followed by RNase digestion at 42°C and gel electrophoresis of the products.

The autoradiogram of the resulting gel is shown in Fig 2. Unhybridized, undigested probe is seen as a single band corresponding to a fragment of the predicted 263 nucleotide size (lane 14), reflecting the fact that there is only one TCRG gene rearrangement containing Vγ1 gene segments in this cell line (Table 1; Tycko and Sklar, unpublished results). When no test RNA was added to the hybridization mixture, the probe is digested completely (lane 13). Test RNA synthesized from pure SUP-B7 DNA yields a variety of protected fragments (lane 1), the largest of which is 244 nucleotides long, corresponding to the size predicted for the probe protected from RNase digestion by perfectly matched test RNA. This band declines in intensity with progressive dilutions of SUP-B7 DNA into controlled bone marrow DNA (lanes 2 through 7) down to a dilution of 10⁻⁵ (lane 6), but cannot be identified in a dilution of 10⁻⁶ (lane 7) or in DNA of normal bone marrows (lanes 8 through 12). These findings are consistent with the maximum theoretical sensitivity of assay, because 1 µg of DNA is the equivalent of 10⁸ cells and a dilution of 10⁻⁶ is therefore expected, on average, to contain only one copy of the tumor-specific TCRG gene rearrangement. The bands observed in the control bone marrow lanes correspond roughly to the length of the Vγ segments within the PCR products. Presence of these bands implies that an efficient transcription of the nonradioactive test RNA had in fact occurred. That multiple bands smaller than 244 nucleotides are observed with pure SUP-B7 test RNA indicates that RNase A may cleave even double-stranded RNA to a certain extent at the digestion temperature used for these assays.

DNA from the cell line SUP-T1 was analyzed in a manner analogous to that with which SUP-B7 was studied (Fig 3). SUP-T1 is known to contain two TCRG gene rearrangements involving Vγ1 gene segments (Table 1), and consequently, the SUP-T1 probe consists of two RNA transcripts resolved as two separate bands in a polyacrylamide gel (lane 14). The major fragment protected from RNase digestion at 45°C results in a band at the position consistent with derivation from the smaller of the two probes. This band could be identified with dilutions of SUP-T1 DNA down to 10⁻³ (lanes 1 through 6), but not with further dilutions (lane 7) or with DNA of normal bone marrow controls (lanes 8 through 12).

In contrast to cell lines, clinical samples are rarely...
composed of pure populations of tumor cells. Since some fraction of rearranged TCRG genes in diagnostic specimens can be expected to represent normal, polyclonal T cells, probe synthesized from tumor samples will often consist of RNA containing sequences different from the tumor-specific TCRG rearrangement(s). It is therefore important to demonstrate that a false-positive signal in the RNase protection assay will not result from RNA which is transcribed from heterogeneous gene rearrangements in normal lymphocytes and which hybridizes without mismatches to test RNA synthesized from the same rearrangements present in normal marrow cells. This issue was addressed in an experiment shown in Fig 4. Probes synthesized from two normal bone marrows were hybridized to test RNAs from the same marrow samples and subjected to RNase A digestion at 50°C. As expected, the probes generated from the two bone marrow specimens are composed of RNA transcripts of varying lengths (lanes 3 and 6), and after digestion of the hybrids, no bands are detectable in the autoradiogram at the positions appropriate for full-length protection of any of the probes (lanes 1 and 4). This result confirms the great diversity of DNA sequences in TCRG gene rearrangements of normal bone marrow.

Another issue related to the heterogeneity of most clinical samples is the potential reduction in the sensitivity of the assay if the diagnostic specimen from which the RNA probe is generated contains highly impure tumor. This problem was investigated by preparing probes from dilutions of cell line DNA in normal bone marrow DNA and performing assays with these probes on test RNA made from dilutions of the respective cell line. These analyses showed that even when the cell line DNA was diluted by a factor of 10⁻³, tumor cells could still be detected at a concentration of 1 in a 1,000—a level at least one order of magnitude more sensitive than routinely achieved by conventional Southern blotting.

To test the applicability of the RNase protection assay for actual tumor specimens, three cases of ALL were examined. These cases had previously been analyzed by PCR amplification using tumor-specific primers complementary to junctional region sequences of rearranged TCRG genes. The first case, from patient A, was previously demonstrated by this method to contain tumor at the level of approximately 10⁻³ cells in a specimen of bone marrow obtained when the patient was believed to be in a complete clinical remission 1 month before a subsequent relapse (pt.5 in ref 7). Figure 5 shows the results of assays with specimens from this case. A single band is seen in the lane containing undigested probe amplified from the original diagnostic specimen (lane 16), suggesting that only one TCRG gene rearrangement was present in the tumor. The position of fully protected probe is illustrated in lane 1, which shows the result with test RNA amplified from the original leukemia DNA (lanes 2 through 8). A fully protected fragment is seen only in one of two independently amplified...
samples of the $10^{-1}$ dilution, suggesting that at this level the assay is at the Poisson limit (lanes 6 and 7). Comparing the intensities of the bands produced from the remission and dilution samples, the fraction of leukemic cells in the remission specimen can be estimated to be about 1% of the total number of bone marrow cells. The specificity of analyses on this case was confirmed by studies on five normal bone marrows from unrelated individuals, none of which showed bands in the position corresponding to fully protected probe (lanes 10 through 14).

The second case of ALL, from patient B, was similar to the first case, except that the level of residual disease determined previously in a presumed remission specimen, obtained 20 months before relapse, was much lower than that in patient A—approximately $10^{-5}$ (pt 6 in ref 7). As shown in Fig 6, only one TCRG gene rearrangement appears to have been present in the tumor (lane 16). Analyses of diagnostic and relapse specimens show bands corresponding to the positions expected for fully protected probe. By comparison to results of dilutions of tumor DNA, residual disease was detected at a level of approximately 0.001%, in agreement with the findings obtained earlier by amplification with junctional region primers.  

The third case of ALL, from patient C (pt 1 in ref 7), was selected for analysis because it contained a TCRG gene rearrangement with an N insertion consisting of only a single nucleotide—a feature that conceivably could reduce the specificity of this junctional region for the malignant clone in this case. In fact, this tumor proved to be the most difficult to assay because low amounts of false-positive signal from normal bone marrow could not be eliminated at any temperature of RNase digestion which did not greatly reduce the true positive signal from dilutions of patient C DNA. The minimum level of patient C DNA that could be detected at the lowest temperature (50°C), which extinguished signals from normal bone marrow DNA, was $10^{-8}$.  

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**Fig 4.** RNase protection assay of test RNA synthesized from two normal bone marrow DNAs with probes synthesized from the same bone marrow DNAs.

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**Fig 5.** RNase protection assay of bone marrow DNA from patient A and normal bone marrow DNAs with a probe synthesized from the diagnostic patient A bone marrow sample. The level of residual disease was quantitated by analysis of $10^{-1}$ to $10^{-8}$ dilutions of DNA from the diagnostic sample into normal bone marrow DNA. The arrow has the same significance as in Fig 2.
However, the reason for the difficulty in this case had to be due to more than the small size of the N insertion in one TCRG gene rearrangement since the tumor of patient C contains a second TCRG gene rearrangement with a six nucleotide N insertion, together with rather large deletions from the ends of the rearranged Vγ and Jγ segments (17 and 16 nucleotides, respectively). Gel electrophoresis of the undigested RNA probe showed that this second rearrangement was indeed transcribed, yet, like probe from the first rearrangement, this second probe also failed to detect tumor cells at dilutions of greater than $10^{-3}$.

**DISCUSSION**

This report describes an RNase protection assay using PCR amplification of antigen receptor gene rearrangements to detect and monitor lymphoid neoplasia. Experiments with dilutions of cell line DNA into the DNA of normal, polyclonal bone marrow indicate that, applied to TCRG genes, the method is sensitive enough to detect one clonal cell in $10^3$ total cells—the theoretical limit of maximum sensitivity achievable with the reaction volumes routinely used. In addition, studies of ALL cases show that the method is generally as sensitive as more laborious methods involving the use of tumor-specific PCR primers for monitoring small amounts of residual disease. Altogether, from acquisition of tissue to final autoradiogram, the assay can be completed in 2 to 3 days.

Our primary motivation for developing the RNase protection assay for detecting lymphoid neoplasia was to provide a simplified and rapid alternative to other methods for highly sensitive detection of lymphoid disease lacking suitable chromosomal translocations. Potential areas of application for these techniques include monitoring of residual disease after therapy, staging of disease (in lymphoma), and detection of minute quantities of disease in particular anatomic sites (for instance, ALL in cerebrospinal fluid). Like the RNase protection assay, all comparable methods rely on the clonotypic nucleotide sequences created at the junction of rearranged gene segments in antigen receptor genes. Most of these methods require knowledge of the junctional sequence in order to construct a complementary oligonucleotide to be used either as a primer or as a probe. This requirement implies additional effort to determine the sequence, usually by direct sequence analysis of a PCR product amplified from a diagnostic tumor specimen or by sequence analysis of the PCR product after molecular cloning in a sequencing vector. The former strategy saves time but may not be feasible if there are two clonal gene rearrangements in a case or if the malignant clone represents a small fraction of the cells within the biopsy specimen. The RNase protection assay completely circumvents sequence analysis since the probe is made directly from the diagnostic biopsy specimen. Furthermore, the number of gene rearrangements present in the diagnostic sample is irrelevant since simultaneous analysis with more than one RNA probe is not only possible but may be desirable.

Another step required by methods using oligonucleotide primers or probes is the construction of tumor-specific oligonucleotides to match the junctional sequence. Such oligonucleotides are easily made with DNA synthesizers, but both the instrument itself and the reagents it uses are expensive. Aside from increased cost, this step carries with it the risk of errors in the sequence of the oligonucleotide synthesized, either because the sequence determined for the junctional region was inaccurate or the synthesizer was improperly programmed.
As mentioned above, one of the advantages of the RNase protection assay over methods using junctional sequence oligonucleotides is the ability to work with impure diagnostic biopsy specimens. Experiments in which RNA probe was made from dilutions of cell line DNA showed that even substantial contamination of clonal gene rearrangements with polyclonal rearrangements only moderately reduces the sensitivity of the RNase protection assay. This feature means that the assay could potentially be applied to primary diagnosis of lymphoid neoplasms by generating probe and test RNA from a single specimen and analyzing hybrids between the two RNA products for RNase resistance. This application is not practical for methods using junctional sequence oligonucleotides.

For reasons described above, we have begun our investigations with the RNase protection assay by studying TCR genes. Analyses using the Southern blot technique show clonal rearrangements of this gene in the great majority of T-cell neoplasms and about two thirds of pre-B cell neoplasms. Recent studies using PCR amplification combined with denaturing gradient gel electrophoresis indicate that the actual frequency of lymphoid tumors with rearranged TCR genes may be even higher. The gene is therefore well suited for monitoring of residual disease in ALL, a neoplasm in which the administration of therapy sufficient for eradication of the tumor must be weighed against the toxicity of the treatment.

In principle, an RNase protection assay similar to that used for TCR genes could be used for any antigen receptor gene having N insertions, as long as consensus primers can be found for accomplishing the amplification of probe and test RNAs. This requirement appears to be met by heavy chain immunoglobulin (IgH) genes in a significant number of B-cell neoplasms and by delta T-cell receptor (TCRD) genes in many T-cell neoplasms. Both genes have already been the subjects of PCR-based methods for detecting residual disease. Rearrangements of IgH genes have been analyzed using oligonucleotides for the V-D-J junctional sequence, while TCRD gene rearrangements have been analyzed by using DNA hybridization probes produced directly from PCR products amplified from diagnostic biopsy specimens. The latter approach, like the RNase protection assay, requires no detailed sequence information about the gene rearrangement under examination; however, a conventional preliminary Southern blot is apparently needed in each case to identify the type of TCRD rearrangement in that case. Furthermore, the method is not transferable to other antigen receptor genes and the TCRD gene is clonally rearranged in relatively few lymphoid tumors than the other antigen receptor genes (Loh and Sklar, unpublished results). Preliminary results using PCR-mediated RNase protection to detect B-cell tumors by means of rearrangements in Ig genes suggest a sensitivity similar to that found in analyses of TCRG gene rearrangements (Veelken and Shear, unpublished results).

An unexplained finding with the RNase protection assay was the reduced sensitivity of the assay applied to one case of ALL (pt C). Because both clonal TCRG gene rearrangements in the case are apparently transcribed into probe, it seems unlikely that the problem is intrinsic to the sequences of the rearranged TCRG genes. Nor is the reduced sensitivity a function of the normal bone marrow DNA used as a diluent; the same effect was found with dilutions using DNA from several different normal bone marrows. Perhaps the results in this case reflect some property of the marrow from this case, which somehow interferes with hybrid formation. Whatever accounts for this reduction in sensitivity does not apparently affect amplification of TCRG genes with a junctional region primer. In any event, the level of sensitivity achieved in this case (10^-3), although lower than usual, still exceeds that of conventional methods for detecting lymphoid neoplasia by at least an order of magnitude. It remains to be seen whether this case is an isolated example of reduced sensitivity. However, even if it is not, analyses of dilutions with tumor DNA on each case offers a way of determining the sensitivity of the RNase protection assay for a particular case and avoiding an inadvertent over-estimation of the sensitivity in that case.

Leaving aside matters of sensitivity, cost, and speed, the overriding issue facing any new diagnostic test is the clinical usefulness of the information provided by it. Experience with tests that detect lymphoid neoplasia at the level of 10^-3 is sufficiently early that the answer to this question is not yet clear. Nevertheless, early indications in ALL are that patients in long-term remission and presumably cured do not have disease detectable at this level, and that at least a significant fraction of patients in remission who later relapse have detectable disease in remission bone marrow. Based on these findings, highly sensitive methods for lymphoid neoplasia seem to hold promise as a valuable tool for the management of these malignancies. A convenient and inexpensive RNase protection assay for this purpose, as described here, should increase the value of this kind of test.

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