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Rapid, nonradioactive detection of clonal T-cell receptor gene rearrangements in lymphoid neoplasms

(γ T-cell receptor gene/lymphoma/leukemia/polymerase chain reaction/denaturing gradient gel electrophoresis)

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ABSTRACT **Southern blot hybridization analysis of clonal antigen receptor gene rearrangements has proved to be a valuable adjunct to conventional methods for diagnosing lymphoid neoplasia. However, Southern blot analysis suffers from a number of technical disadvantages, including the time necessary to obtain results, the use of radioactivity, and the susceptibility of the method to various artifacts. We have investigated an alternative approach for assessing the clonality of antigen receptor gene rearrangements in lymphoid tissue biopsy specimens. This approach involves the amplification of rearranged γ T-cell receptor genes by the polymerase chain reaction and analysis of the polymerase chain reaction products by denaturing gradient gel electrophoresis. By use of this approach, clonal rearrangements from neoplastic lymphocytes constituting as little as 0.1–1% of the total cells in the tissue are detected as discrete bands in the denaturing gel after the gel is stained with ethidium bromide and viewed under ultraviolet light. In contrast, polyclonal rearrangements from reactive lymphocytes appear as a diffuse smear along the length of the gel. Our findings suggest that polymerase chain reaction combined with denaturing gradient gel electrophoresis may offer a rapid, nonradioactive, and sensitive alternative to Southern blot analysis for the diagnostic evaluation of lymphoid tissue biopsy specimens.**

Diagnosis of lymphoid neoplasms is often complicated by difficulties in distinguishing histologically between neoplastic and reactive lymphocytes. In this situation, clonality of the lymphocytes within a biopsy specimen can provide an additional useful criterion for the diagnosis of lymphoid neoplasia. One method commonly used for assessing the clonality of lymphocytes has been the detection of clonal antigen receptor gene rearrangements in biopsy tissues by Southern blot hybridization (1–4). This method of analysis is based on the fact that the DNA of immunoglobulin or T-cell receptor genes within developing B and T lymphocytes undergoes a series of rearrangement events in which individual variable (V), diversity (D), and joining (J) region segments are joined together from multiple dispersed V, D, and J segments encoded within the germ-line form of these genes. In this way, lymphocytes acquire continuous and structurally diverse coding sequences for antigen receptor proteins with specificities for different antigenic determinants.

On a genetic level, the configuration of DNA in the rearranged locus marks the cell in which rearrangement has occurred and any clonal descendants that may arise from that cell. During preparation of a Southern blot, cleavage of DNA in a rearranged antigen receptor gene with an appropriate bacterial restriction enzyme produces a DNA fragment of length dependent on the specific configuration of DNA in the locus. When applied to DNA extracted from homogeneous, clonal populations of lymphocytes, Southern blot analysis detects this fragment as a rearranged band at a position in an autoradiogram characteristic for the clone being analyzed. When applied to DNA of tissue biopsy specimens, Southern blot analysis essentially provides a method for assaying the fraction of cells that contain uniform gene rearrangements and thus belong to the same clone. Detection of rearranged, non-germ-line bands for antigen receptor genes in autoradiograms indicates the presence of a major population of clonal lymphocytes comprising at least 1–5% of the total cells in the specimen (2, 3). Since virtually all neoplastic processes are composed of clonal cellular proliferations, analyses of tissues containing neoplastic lymphocytes yield prominent rearranged bands in the Southern blot autoradiogram. On the other hand, reactive lymphoid processes are generally polyclonal, consisting of cell populations representing numerous, different, and minor lymphocytic clones. Ordinarily, none of these minor clones constitutes >1–5% of the total cells in the specimen, and no rearranged bands are observed in the autoradiogram.

Besides offering information about the neoplastic or reactive nature of lymphoid lesions, Southern blot analyses of antigen receptor gene rearrangements have been helpful in identifying multiple lymphocytic clones or subclones in certain lymphoid tumors (5–7), in assigning B or T lineage to neoplasms of unclear phenotype (8–10), and in monitoring residual disease or relapse after therapy for lymphoid cancers (11, 12). However, despite the variety of applications that Southern blot analysis of antigen receptor genes has found for evaluating lymphoid tissue biopsy specimens, this method suffers from several serious technical disadvantages. One disadvantage relates to the time required to obtain interpretable results (an interval from biopsy to read-out of about 10 days). Additionally, hybridization probes must be labeled with radioactivity, usually ^{32}P, to achieve sufficient sensitivity for most routine purposes. Finally, the Southern blot procedure is subject to certain artifacts, such as nonspecific degradation of DNA prior to or during extraction from the tissues and high background radioactivity adhering to the Southern blot membrane after hybridization.

To circumvent some of the most severe disadvantages of the Southern blot procedure, we have explored an alternative strategy for analyzing antigen receptor gene rearrangements in lymphoid tissue specimens. This strategy uses polymerase chain reaction (PCR) amplification of rearranged antigen receptor DNA within tissues followed by denaturing gradient gel electrophoresis (DGGE) of the PCR products. In the studies described in this report, we have focused on rearrangements of the γ T-cell receptor locus. We have found that

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Abbreviations: PCR, polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; V, variable; D, diversity; J, joining.

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we can obtain the same kinds of information about lymphoid tissue specimens as can be gained from Southern blot analyses of this gene, with at least the same sensitivity, but without using radioactivity and in only 1–2 days.

MATERIALS AND METHODS

Tissues, Cell Lines, and Preparation of DNA. All tissues were obtained with informed consent of the patients. Diagnoses of surgical specimens were established by conventional morphologic methods. The T-lymphoblastic leukemia cell lines SUP-T1 (13) and SUP-T6 (14, 15) were used as a source of homogeneous T-lineage cells for control experiments. Normal thymus was obtained from children undergoing cardiac surgery. Normal bone marrow was obtained from bones removed during surgical procedures (ribs removed during thoracotomies and femoral heads removed for hip replacements) and from donors for allogeneic bone marrow transplantation. Normal blood was obtained from volunteers. Mononuclear cells were separated from bone marrow and blood by sedimentation in gradients of Ficoll–Paque (Pharmacia) (16) and stored at −70°C, as were all solid tissues and cell lines, prior to DNA extraction. DNA was extracted by standard procedures (17) and stored at −20°C.

Southern Blot Analysis. Southern blot analysis for rearrangements of γ T-cell receptor genes was performed as described (1, 2). Five micrograms of DNA was digested with the *Bgl* II or *Bam*HI restriction enzyme (New England Biolabs). The resulting fragments were separated by electrophoresis in a 0.8% agarose gel and then transferred to activated nylon membranes (Genatran; Plasco, Woburn, MA). Membranes were hybridized with a J_γ probe (18) radiolabeled with [α-^{32}P]dCTP by the random priming method (19).

PCR. General procedures for PCR amplification of γ T-cell receptor gene rearrangements have been described elsewhere (20–22). Reactions were performed with 2 μg of total cellular DNA; 300 ng of each of two oligonucleotide primers; 200 μM dATP, dGTP, dCTP, and dTTP; 50 mM KCl; 10 mM Tris·HCl (pH 8.3 at 25°C); 1.5 mM $MgCl_2$; 0.01% gelatin; and 2.5 units of *Thermus aquaticus* DNA polymerase, in a total volume of 100 μl. One pair of oligonucleotide primers (referred to as the "outer" pair) contained the sequences 5′-GAAGCTTCTAGCTTTCCTGTCTC-3′ and 5′-CGTCGACAACAAGTGTTGTTCCAC-3′ (corresponding to conserved V_γ and J_γ sequences, respectively), and the other pair (the "inner" pair) contained the sequences 5′-CTCGAGTGCGCTGCCTACAGAGAGG-3′ and 5′-GGATCCACTGCCAAAGAGTTTCTT-3′ (corresponding to conserved V_γ and J_γ sequences, respectively, immediately internal to sequences complementary to the outer pair of primers). The two J_γ primers anneal to the $J_{\gamma1}$ and $J_{\gamma2}$ segments and can be used to amplify all γ T-cell receptor gene rearrangements. The two V_γ primers anneal to sequences of the $V_{\gamma1-8}$ segments and can be used to amplify the great majority of γ T-cell receptor gene rearrangements found in malignant lymphoid cells (ref. 20 and unpublished observations). The outer pair of V_γ and J_γ primers generates a product of about 500 base pairs (bp); the inner pair generates a product of about 460 bp. The initial denaturation conditions used were 94°C for 3.5 min, with subsequent denaturation in later cycles at 94°C for 1.0 min. Primer annealing in each cycle was performed at 55°C for 1.5 min and strand elongation was at 70°C for 1.5 min throughout. For most analyses, 45 cycles of synthesis were carried out by permitting the reaction to proceed first for 25 cycles, after which 1/10th of the reaction mixture was removed, added to fresh starting reagents, and allowed to polymerize for another 20 cycles. The first set of cycles in all studies was performed with the outer pair of primers. In some analyses, the second set of cycles was performed with the same primers, but in other analyses, the second set of cycles was performed with the inner V_γ and J_γ primers. All PCR reactions were carried out in a Thermal Cycler incubator (Perkin–Elmer).

Radiolabeling of PCR products was carried out by using 5′ end-labeled oligonucleotide primers in the amplification reaction. Oligonucleotides were labeled by incubating 2.5 μg of the J_γ primer for 30 min at 37°C with 50 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP (3000 Ci/mmol) and 10 units of polynucleotide kinase in a total volume of 50 μl containing 0.5 M Tris·HCl (pH 7.6), 0.1 M $MgCl_2$, 50 mM dithiothreitol, 1 mM spermidine, and 1 mM EDTA. After completion of the reaction, the primer was separated from the reaction mixture by centrifugation through a Sephadex G-50 column. The conditions for PCR with the labeled primer were the same as those used with unlabeled primers.

DGGE. DGGE was run in an apparatus purchased from the Green Mountain Laboratory Supply Company (Waltham, MA) following procedures recommended by Myers *et al.* (23). Gels contained 6.5% acrylamide and a 30–60% gradient of urea/formamide denaturing solution [80% denaturing solution: 5.6 M urea/32% (vol/vol) deionized formamide] made up in TAE buffer (0.04 M Tris base/0.02 M sodium acetate/0.001 M EDTA, pH 7.4). The dimensions of the gels were 20 cm × 17 cm × 0.75 mm. Before loading on the gels, PCR products were precipitated in 0.3 M sodium acetate and 70% ethanol at −20°C and resuspended in 10 μl of loading buffer (20% Ficoll/10 mM Tris, pH 7.8/1 mM EDTA/0.5% bromphenol blue). This solution was incubated at 95°C for 5 min and rapidly cooled to and held at 60°C for 1 hr prior to loading on the gels. Gels were run at 150 V in TAE buffer at 60°C with constant recirculation of buffer between the upper (cathodic) chamber and the lower (anodic) chamber. After about 6 hr of electrophoresis, gels were removed from the apparatus and stained by immersion for 15 min in a solution of 1 μg of ethidium bromide per ml in water. Following staining, gels were placed on a 300-nm wavelength UV viewer and photographed with 53 Polaroid film.

RESULTS

Conditions for efficient PCR amplification of rearranged γ T-cell receptor genes from neoplastic tissues with the V_γ and J_γ primers were established in earlier studies (20). These conditions were used to amplify rearranged genes from the SUP-T1 and SUP-T6 cell lines with different primer pairs consisting of the outer conserved J_γ primer and one of several V_γ primers. Products of these reactions were separately run on perpendicular denaturing gradient gels [in which the product is loaded in a broad well along the top of a gel containing a 0–80% gradient of urea/formamide oriented perpendicular to the electrical field (23)]. A clearer transition to a partially denatured state was observed with products obtained from PCR with the outer conserved region V_γ primer described above (data not shown), and subsequent work was carried out with this primer. Results of these studies with the V_γ primer indicated that the two PCR products obtained from SUP-T1 and the two products obtained from SUP-T6 all underwent partial denaturation between about 45% and 55% denaturant. Therefore, 30–60% denaturation gradients were used in later parallel gradient gels.

Various malignant lymphocytic processes, including peripheral T-cell lymphomas, T-cell leukemias, cutaneous T-cell lymphomas, and lymphoblastic neoplasms of T lineage, were selected for analysis. Each of these cases was regarded as unequivocally malignant by morphologic standards. Southern blot analyses were performed to determine the presence of clonal γ T-cell receptor gene rearrangements in these tissues (Fig. 1*A*). DNA from cases showing clonal γ

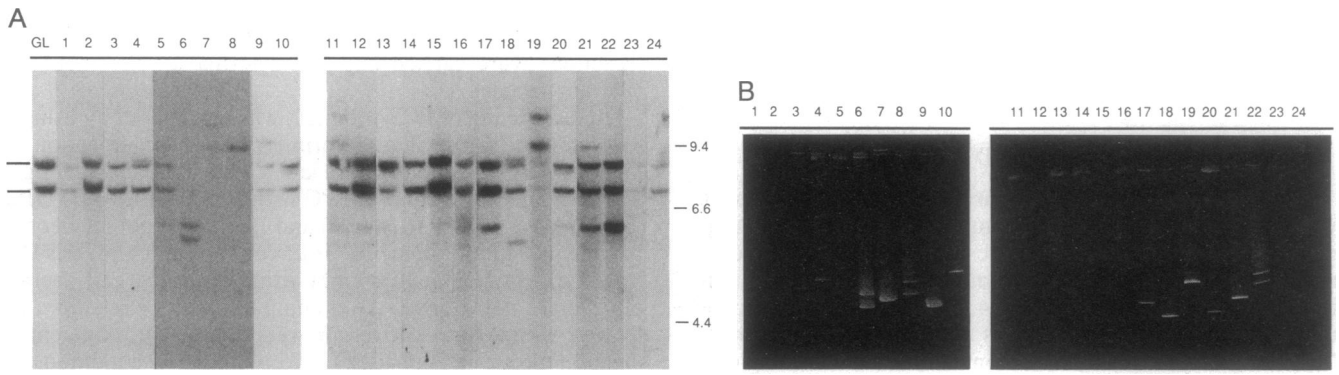

FIG. 1. Analyses of γ T-cell receptor gene rearrangements in malignant and benign tissues. (*A*) Southern blot autoradiograms obtained after digestion of DNA with *Bgl* II restriction enzyme and hybridization with a J_γ probe. Bars at the left indicate the positions of the two unrearranged γ T-cell receptor germ-line bands. The relative positions of size marker fragments [in kilobases (kb)] are shown at the right. Tissues examined include the following: normal sperm (lane GL), Hodgkin disease (lane 1), normal bone marrow mononuclear cells (lane 2), chronic myelocytic leukemia (CML) (lanes 3 and 4), normal peripheral blood mononuclear cells (lane 5), mononuclear cells from bone marrow involved in acute lymphoblastic leukemia (T lineage) (lanes 6–8), human T-lymphotropic virus I-associated adult T-cell leukemia (lanes 9 and 10), hyperplastic thymus (lane 11), reactive spleen (lane 12), hyperplastic tonsil (lane 13), follicular and diffuse small cleaved cell lymphoma (B lineage) (lane 14), reactive lymph node (lane 15), normal thymus (lane 16), diffuse large cell lymphoma (T lineage) (lane 17), lymphoblastic lymphoma (T lineage) (lanes 18 and 19), diffuse mixed lymphoma (T lineage) (lane 20), cutaneous T-cell lymphoma (mycosis fungoides) (lanes 21 and 22), diffuse large cell lymphoma of skin (T lineage) (lane 23), and normal skin (lane 24). Non-germ-line bands seen in analyses of polyclonal tissues are presumably due to frequent independent rearrangements using the same V_γ and J_γ gene segments. (*B*) PCR/DGGE analysis of DNA from the same tissues as in *A*. Two faint but discrete bands were visible in the 50–55% region of the gel in lane 23, along with more intense bands seen in lanes 4, 6–10, and 17–22. PCR for each analysis was performed using only the set of outer V_γ and J_γ oligonucleotide primers.

T-cell receptor gene rearrangements was subjected to PCR amplification and DGGE. At least one band was observed in the gels following ethidium bromide staining for each case previously found to contain clonal rearrangements (Fig. 1*B*), although the intensity of these bands did not necessarily correlate with the relative intensity of bands detected in the Southern blot autoradiogram for that case. Bands in the denaturing gels varied in position from case to case but clustered around the position of about 50–55% denaturant.

A series of benign tissues, including bone marrow, peripheral blood, thymus, lymph node, and skin, along with several tumors that usually lack clonal γ T-cell receptor rearrangements [B-cell lymphomas, Hodgkin disease, and chronic myelocytic leukemias (CML)] were studied as negative controls. Southern blot analyses of these tissues showed only polyclonal patterns of γ T-cell receptor gene rearrangements (see discussion below), except for one case of CML (Fig. 1*A*, lane 4) that showed a single intense rearranged band at the 9.6-kb position. PCR products amplified from this specimen formed two discrete bands in denaturing gradient gels (Fig. 1*B*, lane 4). When PCR products from benign tissues and non-T-cell tumors initially were run directly on denaturing gradient gels, no major bands were detected, but several faint bands were frequently seen at the same approximate positions in the 40–55% denaturing region of the gels for each specimen (data not shown). To eliminate these bands, which often comigrated with bands derived from clonal rearrangements, an additional step of melting and reannealing of the PCR products was introduced prior to loading of the sample on the gels. After this modification, gel analyses of PCR products from tissues lacking clonal γ T-cell receptor gene rearrangements revealed no discrete bands and only a diffuse smear of stained material in a region above the zone in which most bands derived from clonal rearrangements appeared. Inclusion of this step resulted in only a minor reduction in the intensity of bands from clonal rearrangements.

The nature of the diffuse smear seen in most lanes of the gels was investigated by electrophoretic transfer of the gel contents onto nylon membranes, followed by hybridization of the membrane with a 5′ end-labeled V_γ oligonucleotide complementary to conserved sequences 3′ to those corresponding to the V_γ primer used in PCR. Autoradiography of the membrane showed hybridization both to the discrete amplified bands and to full length of the smear, indicating that the smear was composed of amplified γ T-cell receptor DNA.

In addition to discrete bands and/or a smear of stained material, a doublet of bands was often seen at a high position in most analyses of malignant or benign tissue DNA (Fig. 1*B*). The origin of this doublet is unclear. However, the position of this doublet is well above that of the highest bands obtained from malignant specimens and therefore does not interfere with interpretation of the results. Furthermore, the presence of this doublet serves as a control for the quality of the template DNA to confirm that amplification is possible even if no bands corresponding to clonal γ T-cell receptor rearrangements are seen in the gel.

To determine the sensitivity of PCR combined with DGGE for detecting clonal γ T-cell receptor gene rearrangements, mixtures of cell line DNA with various proportions of DNA from polyclonal tissue (normal bone marrow) were analyzed (Fig. 2*A*). After 45 cycles of PCR, clonal bands were obtained from mixtures containing as little as 1–0.1% cell line DNA in bone marrow DNA. Additional cycles of amplification did not appear to increase the sensitivity of the procedure.

Although one of the primary goals of this research was the development of a nonradioactive method for detecting clonal antigen receptor gene rearrangements in tissue specimens, we were curious to know whether levels of sensitivity even greater than that demonstrated by ethidium bromide staining of gels could be achieved by incorporating radioactivity into the PCR amplification product. For this purpose, the 5′ end of the J_γ oligonucleotide primer was labeled with ^{32}P prior to PCR on the mixtures of cell line DNA with bone marrow DNA described above. Electrophoresis of the PCR products on a denaturing gradient gel followed by autoradiography revealed definite bands down to a dilution of 0.1% cell line DNA in bone marrow DNA (Fig. 2*B*).

DISCUSSION

Southern blot hybridization and PCR/DGGE applied to the clonal analysis of lymphoid tissue biopsy specimens rely on the detection of clonally specific markers within the DNA of

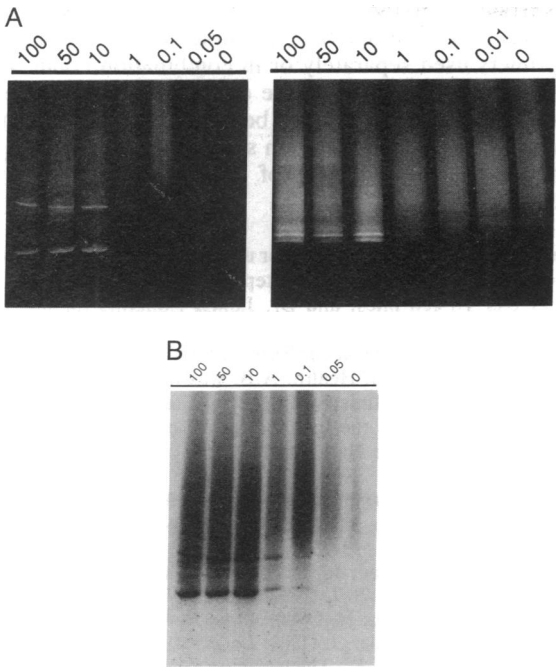

FIG. 2. Determination of sensitivity of PCR/DGGE analysis for detecting clonal γ T-cell receptor gene rearrangements in tissue DNA. (*A*) Gels stained with ethidium bromide and illuminated with UV light. Analyses were carried out on DNA from T-cell leukemia cell lines diluted into DNA of normal bone marrow mononuclear cells. Dilutions on the left were made with SUP-T1 DNA and those on the right with SUP-T6 DNA. Numbers at the top indicate the percent of cell line DNA in a total of 2 μg of DNA used for each PCR reaction. PCR was performed for 25 cycles using the outer V_γ and J_γ primers followed by 20 cycles using the inner V_γ and J_γ primers. (*B*) Autoradiogram of gel on left in *A*, which was prepared with radiolabeled PCR products.

rearranged antigen receptor genes. Both methods offer a means for assessing the frequency of this marker in the total DNA of these specimens and therefore determining the abundance within the tissues of the clonal cells that bear this marker in their DNA. However, the precise nature of the marker differs between the two techniques. As described above, the marker analyzed in Southern blot hybridization is the configuration of DNA for rearranged V, (D), and J gene segments, reflected in the size of a DNA restriction fragment produced from the rearranged locus. In PCR/DGGE, all of the DNA fragments generated by PCR and applied to the gel are similar in size—in the present study, about 500 or 460 bp, corresponding to the distances between the positions of the V_γ and J_γ primer sequences in rearranged γ T-cell receptor genes. In this method, the marker is the specific sequence of base pairs in the PCR products amplified from the rearranged genes. Small domains within double-stranded fragments of DNA melt at differing positions along the denaturing gradient of the gel depending on the exact sequence of base pairs within each domain. Regions of single-strandedness within the fragment significantly reduce the mobility of the fragment in the gel, creating bands at varying positions. If sufficient numbers of the PCR products have domains of identical sequence that show the same pattern of melting in the denaturing gel, a band will be seen. Under the conditions used in our studies, this number of PCR products is obtained from about 0.1–1% of the total cells.

The specific differences in the sequences of PCR products that account for the variable positions of clonal bands in our denaturing gels are not known. It seems unlikely that the major factor controlling these positions is DNA sequence in either the V or J region segments of the γ T-cell receptor gene, since the number of band positions detected from clonal tissues and the diffuseness of the smear observed from polyclonal tissues seem inconsistent with the small number of V and J region segments available in this gene. It seems more likely that sequences in the junctional regions between rearranged V and J regions are the principal determinants of the positions of bands. These regions show small, variably sized deletions of germ-line sequence at the 3′ and 5′ ends of the rearranged V and J regions plus the insertion of random base pairs (so-called N sequences) between the fused ends of the two rearranging segments (24, 25). The combined result of these changes is a stretch of highly variable sequence specific for a given locus, cell, or clone.

Some investigators have argued that the simple structure of the γ T-cell receptor locus renders this gene less useful for Southern blot analysis of tissue biopsy specimens than are other antigen receptor genes (26, 27). These investigators reason that even polyclonal populations of lymphocytes are apt to display discrete bands because the paucity of functional V and J segments (about 10 and 2, respectively) leads to frequent joining of the same V–J segments. This phenomenon can be seen in Fig. 1*A* (for example, lanes 11–16), although the extent to which it is true seems to depend on the restriction enzyme used in the analysis (18). The same considerations, however, are not relevant to analysis of the γ T-cell receptor gene by PCR/DGGE. Existing data are insufficient to quantitate accurately the diversity of the junctional sequences on which the separation of PCR products seems to be based, but diversity of sequence in this region probably adds at least several orders of magnitude in variability to the combinatorial diversity of V–J joining in this gene (25). With the assumption that DGGE can detect a single base pair difference in the domain of denaturation, as has been shown in other genes (28, 29), the cumulative effect of sequence variation at the V–J joint would suggest that PCR products derived from any two γ T-cell receptor gene rearrangements should rarely band in exactly the same positions in denaturing gradient gels.

Analysis of T-cell receptor gene rearrangements in tissue biopsy specimens by PCR/DGGE has several advantages over conventional Southern blot hybridization for the same purpose. First, it is much faster, providing results in 1–2 days, as opposed to the 1–2 weeks required for Southern blot analysis. This is particularly important when considering the frequency with which Southern blots must be repeated due to poor results or because of the desirability of repeating the analysis with a different restriction enzyme or hybridization probe to confirm an earlier result. Second, analysis of gene rearrangements by PCR and DGGE does not require radioactivity. This feature reduces the cost, avoids the labor involved in the repeated radiolabeling of probes, and eliminates the inconvenience and expense of proper disposal of radioactivity and contaminated materials.

The PCR/DGGE approach to gene rearrangement analysis also appears to be less susceptible to artifacts often associated with Southern blot hybridization. For example, radioactivity that binds nonspecifically to Southern blot membranes frequently creates shadows and smudges in the autoradiograms, potentially obscuring rearranged bands. Nothing comparable to this problem occurs in analyses using PCR/DGGE. Another artifact in Southern blot analysis is the possible superimposition of rearranged and germ-line bands in the autoradiogram arising from the chance coincidence in the sizes of germ-line and rearranged restriction fragments—a possibility that increases with the use of enzyme-probe combinations that produce many germ-line fragments or when germ-line and rearranged fragments are relatively large in size, due to the diminished resolution of larger fragments in agarose gels. This problem is obviated by the

lack of germ-line bands in analysis of gene rearrangements by PCR/DGGE.

Other advantages of PCR/DGGE relate to the small region of DNA (<500 bp) that constitutes the target for analysis. As a result, PCR products can be obtained from partially degraded DNA most of the time. On the other hand, degradation can be a major problem in Southern blot hybridization, in which the analysis is based on detection of DNA fragments between about 1 and 25 kilobases in length, and usually in the 3- to 23-kb range. The effects of degradation in Southern blot autoradiograms are to reduce the intensity of the rearranged band and, probably even more importantly, to increase a diffuse background signal in the lane. Although extensive degradation may reduce the intensity of bands produced by PCR/DGGE, it does not increase the background and thus cannot potentially mask a faint band that would be otherwise detected. Moreover, PCR/DGGE avoids problems associated with the detection of rearrangements in very large or very small DNA fragments by Southern blot analysis. These rearrangements present no special difficulty for PCR/DGGE, but are sometimes missed in Southern blot analyses because either the large fragments transfer incompletely from gels to membranes or the small fragments are run off the end of the gel prior to transfer.

PCR/DGGE may also have advantages over Southern blot analysis of antigen receptor gene rearrangements in terms of sensitivity. Southern blot analysis seems to have an absolute threshold of 1–5%. Using PCR/DGGE, we have achieved a sensitivity of 0.1–1%. Furthermore, by labeling the 5′ end of one of the oligonucleotide primers with ^{32}P and autoradiographing the denaturing gel after electrophoresis, it is generally possible to increase the sensitivity reproducibly down to 0.1%.

In addition, certain clinical situations may also occur in which PCR/DGGE can be applied but the numbers of lymphoid cells in the specimen are inadequate for analysis by Southern blot hybridization. About 10,000 clonal lymphoid cells are necessary to produce a rearranged band in a Southern blot analysis for an antigen receptor gene. In early cutaneous T-cell lymphoma or lymphoblastic leukemia involving the central nervous system, neoplastic cells may be present in a skin biopsy specimen or sample of cerebrospinal fluid at levels well below this number. Unlike Southern blot hybridization, PCR/DGGE offers the potential of analyzing very small specimens in which the amount of lymphoid cells may be only 100 or less (data not shown).

In this report, PCR/DGGE has been described for clonal analysis of lymphoid tissue specimens using the γ T-cell receptor gene. Clonal rearrangements of this gene have been reported to be present in almost all T-lineage neoplasms, in about two-thirds of pre-B-cell leukemias, and in occasional myeloid leukemias (30, 31). These findings indicate the potential utility of this gene for the evaluation of lymphoid tissue specimens. Furthermore, the frequency with which this gene is rearranged in human neoplasms may have been underestimated in the past because of the technical problems associated with detection by Southern blot hybridization. For instance, we have encountered several cases in which PCR/DGGE has detected clonal rearrangements that could not be found by Southern blot analysis of the same tissues.

In principle, PCR/DGGE should be applicable to the study of other antigen receptor genes besides the γ T-cell receptor gene, provided that PCR can be performed efficiently on these genes. The γ T-cell receptor gene has an obvious advantage in this respect because of the structural simplicity of the gene. The δ T-cell receptor gene is also simple and should be amenable to the same type of analysis. With more complex genes, it may still be possible to find regions of conservation among many V and J region segments, as has been reported for the immunoglobulin heavy chain genes that are rearranged in the cells of many acute lymphoblastic leukemias (32). Alternatively, multiple sets of oligonucleotide primers used separately or in combination could serve for amplification of other, more complex genes. The feasibility of this strategy may also be enhanced by finding that certain families of V or J region segments are preferentially rearranged in different types of lymphoproliferative disorders.

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