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Pseudo-Spikes Are Common in Histologically Benign Lymphoid Tissues

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T cell receptor gene rearrangement is a classic marker of T cell clonality and is a useful adjunct in the diagnosis of T cell lymphomas and leukemias. Rearranged V-J gene segments amplified by polymerase chain reaction (PCR) are traditionally analyzed by polyacrylamide gel electrophoresis. We and others have analyzed TCR-γ PCR products using capillary gel electrophoresis, which produces single nucleotide resolution and provides improved diagnostic sensitivity over conventional methods. However, with this marked increase in resolution and sensitivity, it is necessary to re-define normal variation of TCR-γ gene rearrangement in control tissues to allow appropriate interpretation of monoclonality if present. Using DNA capillary gel electrophoresis, we examined the spectrum of normal patterns for TCR-γ in a variety of T-cell-rich, histologically benign tissue types, including spleen, lymph node, tonsil, and blood, and compared this with the patterns in T cell lymphoma samples. We defined relative peak heights as h_1/h_2, where h_1 represents the peak height of the largest peak above the normally distributed population, and h_2 represents the peak height of the normally distributed curve. We found spikes in almost 20% of histologically benign samples with relative peak heights that were more than 0.5 and up to 1.5. We designated these as pseudo-spikes, because they may be mistaken for monoclonal spikes. In contrast, the relative peak height of the T cell lymphoma samples that showed clonal rearrangement was much higher than that of the pseudo-spikes, being at least 2 in 11/11 and at least 3 in 10/11 cases. Our data suggest that peaks with relative height of at least 3 represent a true clonal population in diagnostic samples. Peaks with relative heights of less than 1.5 may be insignificant, while peaks with relative heights between 1.5 to 3 may warrant further evaluation. Although capillary gel electrophoresis is superior in assessing T cell clonality, caution must be exercised when interpreting results, because pseudo-spikes appear to be common in benign tissues with lymphoid populations and are not necessarily indicative of clonal malignant T cell population. ***(J Mol Diag 2000, 2:145–152)***

T cells, the mediators of cellular immunity, bear T cell receptors (TCR) on their surfaces that recognize and bind to specific antigens. These receptors are unique to each T cell clone, and demonstrate great diversity through rearrangement of germline gene segments during maturation.[1,2] In the germline unrearranged state, these genes consist of many different variable (V), diversity (D), joining (J), and constant (C) gene segments. During the process of rearrangement, V, D, J, and C segments are juxtaposed. As the segments become juxtaposed, some nucleotides are excised, and a variable number of nucleotides are randomly inserted. Rearrangement provides diversity, but also permits the variably rearranged TCR genes to serve as unique markers of individual T cell clones. The T cell receptor is a heterodimer, with the majority of T cells bearing the αβ- and a small minority bearing the γδ-heterodimer. During T cell development, TCR-γδ gene rearrangement occurs first, and only if the γδ rearrangement is not productive does rearrangement of αβ occur. Thus, almost all T cells have rearranged TCR-γ genes at the molecular level even if the cell ultimately becomes TCR-αβ-bearing.[3] The TCR-γ gene is also advantageous for analysis because it has a relatively small number of somewhat homologous V and J segments, making it simpler to analyze compared to the other TCR genes.[4] TCR-γ gene rearrangement is thus the most commonly analyzed marker of T cell clonality.

Historically, analysis of TCR gene rearrangement was done by Southern blotting, though it is now most commonly analyzed by polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE).[5–10] The presence of a distinct band of a single size indicates clonality, whereas diffuse staining spanning the expected PCR product size range indicates a heterogeneous collection of amplicons, consistent with polyclonality. However, several technical problems exist with these analyses. Standard-sized polyacrylamide gels lack single basepair resolution, and the distinction between single bands representing monoclonality and diffuse staining indicative of polyclonality can be subtle and interpretation can be somewhat subjective. Furthermore, standard

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PAGE lacks the resolution to detect oligoclonality or biclonality when the clones in question have rearranged gene products that differ in size by only a few basepairs. These patterns may appear erroneously as monoclonal spikes. PAGE is also not sensitive in detecting small populations of clonally rearranged cells in a polyclonal background. Finally, sizing of PCR products on PAGE is relatively imprecise, as it depends on rough extrapolation using adjacent size standards.

Capillary gel electrophoresis (CGE) with automated fluorescent fragment analysis has been used in the analysis of TCR gene rearrangement.[11,12] This technique produces single nucleotide resolution and allows objective quantification of the length distribution of PCR products present. As such, CGE may offer improved sensitivity in the analysis of TCR gene rearrangement and has potential advantage in being a quantitative or semiquantitative tool to detect small populations of clonal cells. It may be useful in the detection of minimal residual disease in T cell lymphomas or leukemias, and may also be a potential tool for early detection.

We assessed the sensitivity of CGE in detecting small amounts of T cell clones in a background of polyclonality and compared this to analysis by PAGE. As CGE has markedly increased sensitivity and nucleotide resolution, we found it necessary to redefine the spectrum of normality in various lymphoid tissues to allow appropriate interpretation of CGE-based TCR clonality assays. In several histologically benign tissues, we have found peaks that could be misinterpreted to indicate monoclonality. We have designated these as pseudo-spikes. By comparing the relative peak heights of pseudo-spikes in benign tissues to the true spikes found in known T cell lymphomas, we attempt to define a threshold to differentiate between non-neoplastic and neoplastic T cell populations.

Materials and Methods

Samples and Patients

We examined the pattern of TCR-γ rearrangement in benign peripheral blood samples, spleens, tonsils, and lymph nodes. The blood samples were discarded specimens that had been submitted to the laboratory for determination of Factor V Leiden status, from individuals who have no clinical evidence of hematological malignancies that would cause clonal rearrangement of their T cell receptor genes. The other tissue specimens were obtained from paraffin blocks. Pathological diagnoses were obtained from the hospital pathology database, and the histology of the tissue blocks was reviewed and confirmed by an independent hematopathologist (F. K. R.). The spleens were histologically benign and had been removed for various indications, including traumatic injury, congestive splenomegaly, adenocarcinoma of the pancreas, and chronic pancreatitis. Tonsil tissue was obtained from tonsillectomy specimens resected for tonsillitis. The lymph nodes were obtained from axillary node dissections performed at mastectomy for breast cancer and were uninvolved by tumor. In addition, pathology specimens with histological diagnosis of T cell lymphoma were analyzed to allow comparison to the benign samples. The diagnosis of T cell lymphoma was based on the combination of morphology, flow cytometry, and immunohistochemistry.

Serial dilutions of DNA derived from a T cell line (Jurkat) and a normal population of T cells from a peripheral blood sample were mixed at various ratios to compare the limits of detection between conventional PAGE and CGE. The polyclonal T cells were enriched from peripheral blood nucleated cells by positive selection of CD2+ T cells with Dynabeads M-450 Pan-T (CD2, Dynal Inc., Lake Success, NY) and then eluted.

PCR

PCR was carried out essentially as described by Benhattar et al with minor modifications.[5] Briefly, a pair of consensus primers was used (forward primer TVγ, 5′-AGGGTTGTGTTGGAATCAGG-3′, reverse primer TJγ, 5′-CGTCGACAACAAGTGTTGTTCCAC-3′) directed at the 3′ end of the Vγ segments and the 3′ end of the Jγ segments, respectively. This primer pair detects 70 to 80% of TCR-γ rearrangements and results in PCR products ranging in size from 160 to 190 bp. The forward primer was fluorescently labeled on the 5′ end with 5-FAM (blue). The reaction was pseudo-nested; the first reaction was carried out with only the reverse primer resulting in linear amplification and the second reaction with both primer pairs, resulting in exponential amplification. This procedure results in similar levels of PCR amplification, increases specificity, and reduces the number of false negative results.[5] The first reaction was composed of a total volume of 20 μl, comprised of 1× PCR buffer (Perkin-Elmer, Foster City, CA), 0.2 μl 1% gelatin, 3 pmol reverse primer, 2.5 nmol dNTP, 0.5 U AmpliTaq Gold (Perkin-Elmer), and 150 ng DNA. Amplification was carried out in the Omnigene Hybaid thermocycler using the following conditions: 94°C for 9 minutes, followed by 25 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 73°C for 30 seconds. The second PCR reaction was standard bidirectional PCR, performed with both TCR-γ primers. β-Globin PCR amplification was multiplexed into this reaction as an internal control to confirm the presence of amplifiable DNA. A 20.6-μl volume of second reaction mix was added to the 20 μl from the first reaction in the same tube, making a total final volume of 40.6 μl. The second reaction volume consisted of 1× PCR buffer, 0.2 μl 1% gelatin, 7.5 nmol dNTP, 20 pmol 5-FAM-labeled TCR-γ forward primer, 17 pmol TCR-γ reverse primer, 7.5 pmol TET-labeled β-globin forward primer (green, 5′-CAACTTCATCCACGTTCACC-3′), 7.5 pmol β-globin reverse primer (5′-GAAGAGCCAAGGACAGGTAC-3′), and 0.5 U AmpliTaq Gold. The forward and reverse primers for both primer sets were at a 1:1 molar ratio in the final volume, and the TCR-γ and β-globin primers were at a 2.7:1 molar ratio. The β-globin primer pair amplifies a 265-bp PCR product. For the second PCR reaction, the samples were denatured at 94°C for 5 minutes, followed

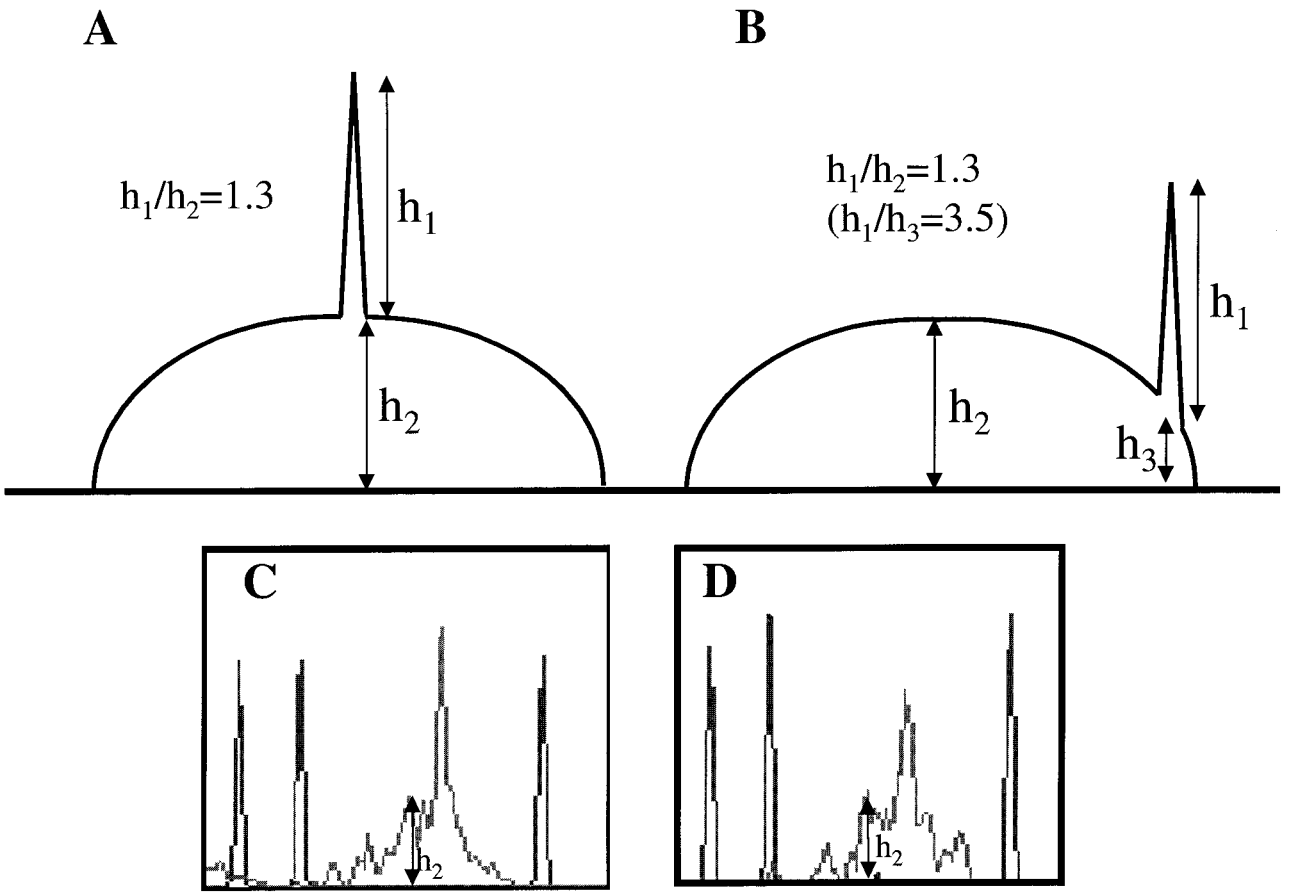

Figure 1. Definition of relative peak heights. Relative peak heights were expressed as h_1/h_2, where h_1 represents the peak height of the largest peak above the normally distributed population, and h_2 represents the peak height of the normally distributed curve. **A** and **B** show h_1/h_2 to be the correctly expressed relative peak height even when the peak in question is displaced toward the edge of the normally distributed population of molecules (**B**). **B** illustrates an alternative ratio (h_1/h_3) where measurement of the peak height of the polyclonal curve was made at the position of the largest peak above the normally distributed population (h_3). This produces a falsely elevated ratio that may be misinterpreted as clonal. **C** and **D** illustrate how h_2 is measured in an actual sample where the polyclonal curve often has jagged edges.

by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 73°C for 30 seconds, and completed with a 5-minute extension at 73°C.

PAGE and CGE

The PCR products were analyzed both on PAGE with ethidium bromide staining and by CGE with automated fluorescent fragment analysis on the ABI 310 genetic analyzer (Perkin-Elmer). For the automated fluorescent fragment analysis, 2 μl of the PCR products was mixed with 12 μl deionized formamide and 0.5 μl of the internal size standard GeneScan Tamara (red, Perkin-Elmer). After denaturation for 5 minutes at 95°C, PCR products were size separated on the genetic analyzer using GeneScan Performance Optimized Polymer 4C (Perkin-Elmer) and analyzed, and size was determined by automated fluorescence quantification using the GeneScan software. When the peak height of amplified PCR product was off scale in the electropherogram to the point where it could not be interpreted, the height limit setting was re-adjusted accordingly on the GeneScan software. If the peak height was still off scale despite the maximum setting allowed on the software, the same PCR product was rerun using a shorter injection time; failing this, less PCR product was loaded for a rerun.

Results

Pseudo-Spikes Are Present in Histologically Benign Samples

We examined the pattern of TCR-γ rearrangement in 22 tonsils, 14 spleens, 9 lymph nodes, and 8 blood samples, all of which were histologically benign. β-Globin amplification was obtained for all samples examined, confirming the presence of amplifiable DNA. All 53 samples showed polyclonal rearrangement on PAGE. When the samples were analyzed using CGE, 50/53 samples showed a polyclonal distribution and 3/53 samples showed an oligoclonal distribution. We noted measurable narrow peaks 1 to 2 bp in width above the polyclonal curve in 14/50 samples. We defined relative peak heights as h_1/h_2, where h_1 represents the peak height of the largest narrow peak above the normally distributed population and h_2 represents the peak height of the normally distributed curve (Figure 1). When the spike is located at the

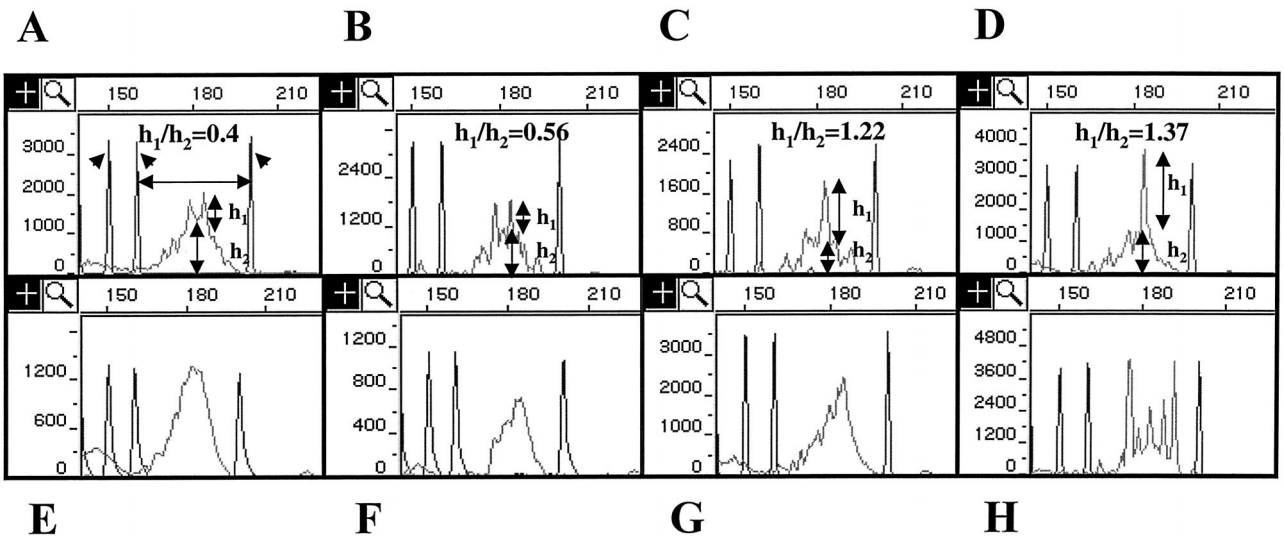

Figure 2. Chromatographs from benign lymphoid tissues. The *y* axis is relative fluorescent units and the *x* axis is size in bases. Size standards corresponding to 150, 160, and 200 bases are shown by **arrowheads** and the area of TCR-γ amplification is defined by the horizontal **double-headed arrow**. **A:** Tonsillitis specimen demonstrating noise. Spleen with capsular laceration and reactive follicular hyperplasia. (**B**), spleen with capsular hematoma. (**C**), and histologically normal spleen from patient with chronic pancreatitis and pseudocyst (**D**), demonstrating pseudo-spikes. Classic bell-shaped curve (**E**), skewed bell-shaped curve (**F**), and bell-shaped curve with sharp peak (**G**), demonstrating range of polyclonal electropherograms. **H:** Oligoclonal pattern in a normal spleen sample from a patient with adenocarcinoma of the pancreas.

edge of the polyclonal curve, h_2 is still defined as the peak height of the normally distributed curve rather than the height of the polyclonal curve at the position of the spike (h_3) to avoid a falsely elevated ratio (Figure 1B). A measurable h_1/h_2 that is <0.5 was noted in 4 tonsil samples but in none of the other three tissue types. We considered peaks where h_1/h_2 was <0.5 to be noise (Figure 2A), and peaks larger than these to have possible significance. Unexpectedly, peaks with h_1/h_2 >0.5 were noted in 10/53 samples (19%), and were found across all four tissue types analyzed. These included 6/14 (43%) spleens, 2/22 (9%) tonsils, 1/9 (11%) lymph nodes, and 1/8 (13%) blood samples. Because these resembled the spikes seen in monoclonal populations, we designated these as pseudo-spikes (Figure 2, B-D). The relative peak heights ranged from 0.56 to 1.37 (Table 1). Although pseudo-spikes were observed in all four lymphoid tissue types studied, they were most prevalent among the spleen samples. The most extreme pseudo-spikes were seen in two spleen samples, the first from a patient with chronic pancreatitis (ratio 1.37) and the second from a patient with carcinoma of the pancreas (ratio 1.28). For both samples, DNA was re-isolated from the paraffin blocks and the analysis repeated to exclude technical error. Similar pseudo-spikes with the same size as previously recorded were found in both samples.

Table 1. Samples Demonstrating Pseudo-Spikes

Sample	Histological diagnosis	h_1/h_2
Spleen	Capsular laceration	0.56
(*n* = 6/14, 43%)	Chronic pancreatitis with pseudocyst	1.37
	Pancreatic adenocarcinoma	1.28
	Capsular hematoma	1.22
	Pancreatic adenocarcinoma	0.82
	Pancreatic islet cell tumor	0.63
Tonsil	Tonsillitis	0.90
(*n* = 2/22, 9%)	Tonsillitis	0.77
Lymph node (*n* = 1/9, 11%)	Histologically normal	0.77
Blood (*n* = 1/8, 13%)	Not applicable	1.10

Spikes in T Cell Lymphoma Samples May Be Distinguished from Pseudo-Spikes in Benign Samples Using Relative Peak Heights

We examined the pattern of TCR-γ rearrangement with CGE in 13 samples with a histological diagnosis of T cell lymphoma: 4 bone marrow, 4 lymph node, 1 spleen, 1 colon, 1 testis, 1 lip, and 1 buccal mass. All 13 samples amplified for the β-globin control. Eleven of 13 samples demonstrated clonal TCR-γ rearrangement, 8 of which were monoallelic and 3 biallelic. The relative peak height was at least 2 in all 11 cases (Figure 3 and Table 2). Two of the 13 samples showed a polyclonal TCR-γ gene rearrangement. This is consistent with the limited sensitivity of the consensus primer pair used in this study to detect all possible TCR-γ gene rearrangements. Enhanced sensitivity may be achieved with additional primer pairs or by combined TCR-β and TCR-γ assays.[13] When comparing PAGE and CGE in analyzing the T cell lymphoma cases, we found concordance between PAGE and CGE in 9/13 cases (6 monoallelic, 2 biallelic, 1 polyclonal). In the 4 cases that were discordant, CGE appears to provide more information than PAGE, showing monoclonal and polyclonal rearrangement, respectively, for two samples for which no bands were detectable on PAGE. For the remaining two samples, CGE detected biallelic and monoallelic rearrangement, *versus* monoal-

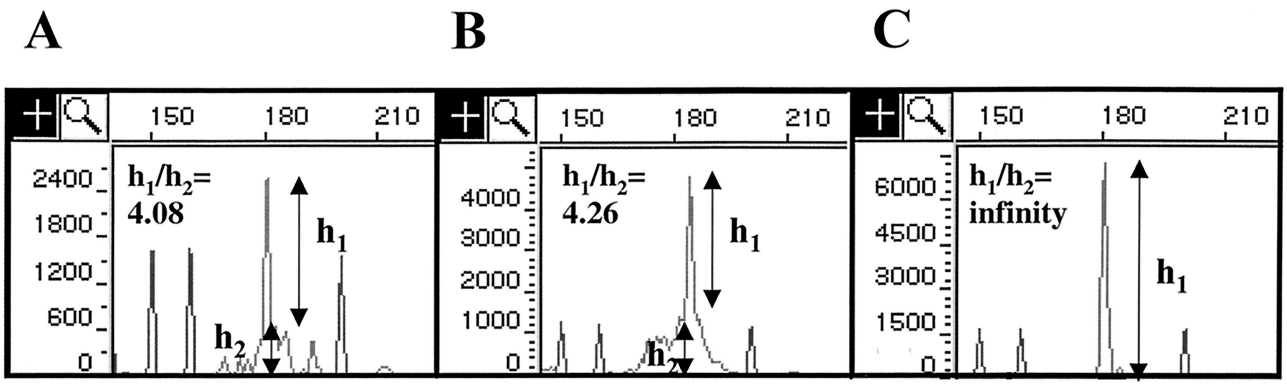

Figure 3. Chromatographs from T cell lymphoma samples demonstrating monoclonal spikes. **A:** Bone marrow with peripheral T cell lymphoma. **B:** Lymph node with peripheral T cell lymphoma. **C:** Bone marrow with peripheral T cell lymphoma. Symbols and axes as in Figure 2.

lelic and polyclonal rearrangement on PAGE, respectively.

Limit of Detection of CGE Was Superior to PAGE

We compared the limit of detection of CGE and PAGE by titrating a T cell lymphoma cell line DNA (Jurkat) into DNA isolated from polyclonal T cells. DNA mixes using Jurkat: normal ratios of 1:10, 1:25, 1:100, and 1:250 were analyzed. We noted CGE to be superior to PAGE in its limit of detection. Using a relative peak height of at least 3 as a threshold, CGE was able to detect quite definitively the presence of the Jurkat DNA in the 1:10 and 1:25 ratio samples, but not in the 1:100 and 1:250 ratio samples (Figure 4B). In contrast, when the same DNA samples were analyzed with PAGE, it was not possible to be certain of the presence of monoclonal DNA even in the 1:10 ratio sample (Figure 4A).

Most Histologically Benign Samples Demonstrate a Polyclonal Spectrum

When the PCR products were analyzed using conventional PAGE, a diffuse staining in the size range of 160–190 bp, indicative of heterogeneous lengths of molecules, was noted in all of the histologically benign samples. When analyzed using CGE, the majority of histologically benign samples demonstrated the expected normally distributed population of products consistent with polyclonality. However, several distinct morphologies of the polyclonal curve were noted. The spectrum varied from the classical bell-shaped curve, to skewed curves and curves that tapered off to a sharp peak (Figure 2, E-G). In addition, we observed an oligoclonal pattern in 1/14 spleen, 1/9 lymph node, and 1/8 blood samples (Figure 2H). This finding is not surprising, given the superior resolution provided by CGE, although its clinical significance is uncertain.

We also analyzed 15 normal skin samples but failed to achieve amplification for TCR-γ in any of the samples. This negative result is possibly due to the scarcity of T cells in normal skin.

Discussion

We examined the polyclonal pattern of a variety of T-cell-rich tissue types, including blood and lymph node samples, on which T cell clonality assays are commonly performed. Somewhat disturbingly, we found peaks higher than could be attributable to noise to be present in almost 20% of the samples. The height ratio of these

Table 2. TCR-γ Gene Rearrangement with CGE in T Cell Lymphoma Samples

Sample	Histological diagnosis	PAGE diagnosis	CGE diagnosis	h_1/h_2
Bone marrow	Peripheral T cell lymphoma	Monoallelic	Monoallelic	∞
Bone marrow	Peripheral T cell lymphoma	No band	Monoallelic	4.08
Bone marrow	T cell lymphoma	Monoallelic	Monoallelic	5.62
Bone marrow	T cell lymphoma	Biallelic	Biallelic	7.68
Lymph node, peritoneal	Peripheral T cell lymphoma	Monoallelic	Monoallelic	∞
Lymph node, retroperitoneal	Peripheral T cell lymphoma	Monoallelic	Monoallelic	4.26
Lymph node, axillary	Peripheral T cell lymphoma, possibly angioimmunoblastic	Biallelic	Biallelic	2.08
Spleen	Hepatosplenic lymphoma	Monoallelic	Biallelic	15.3
Testis	High grade T cell lymphoma	Monoallelic	Monoallelic	∞
Lip mass	Peripheral T cell lymphoma	Polyclonal*	Monoallelic	3.15
Buccal mass	Malignant lymphoma	Monoallelic	Monoallelic	∞
Lymph node	Angioimmunoblastic T cell lymphoma	Polyclonal	Polyclonal	N.A.
Colon	T cell lymphoma	No band	Polyclonal	N.A.

*Suggestion of a monoclonal band amidst a diffuse polyclonal staining.

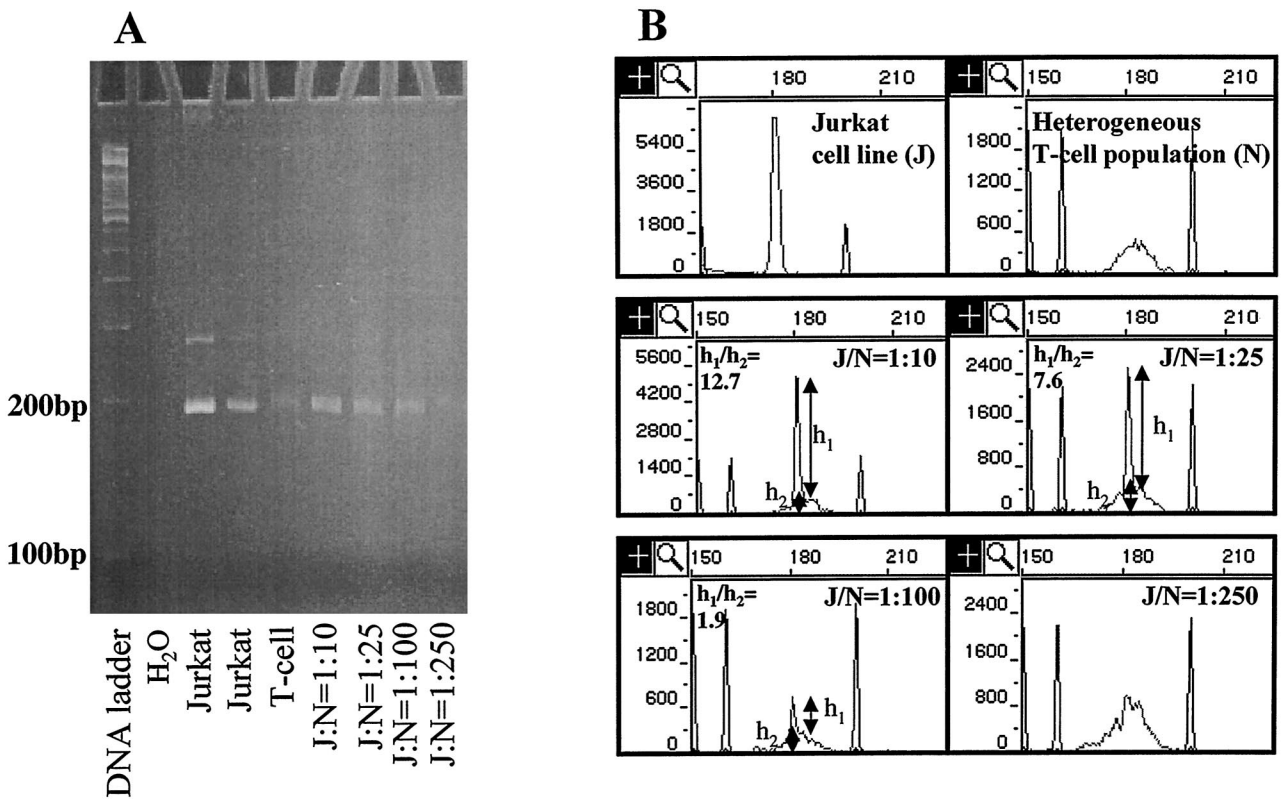

Figure 4. CGE *versus* PAGE in detecting small clonal populations. We mixed varying proportions of a monoclonal population (Jurkat cell line, J) into a heterogeneous polyclonal T cell population (N) and analyzed the mixes with PAGE (**A**) and CGE (**B**). Symbols and axes as in Figure 2. In **A**, the fourth lane from the left shows TCR-γ amplification products from the Jurkat cell line, and the adjacent third lane shows TCR-γ and β-globin PCR products amplified in a single multiplexed reaction from the cell line. With CGE, the presence of the monoclonal population is definitively detected at 1:25 J:N dilution, whereas the presence of a monoclonal band could not be definitively ascertained on PAGE even at 1:10 J:N dilution.

peaks could be surprisingly high (up to 1.37), with the most dramatic cases reminiscent of clonal cells amid a polyclonal population. Because these spikes could be misinterpreted as representing clonality, we designated them as pseudo-spikes.

Pseudo-spikes are clearly common in benign lymphoid tissues when analyzed by CGE and are not indicative of a clonal malignant T cell population in diagnostic samples. They appear to be particularly common in spleen samples, being present in almost half of the samples analyzed. Caution needs to be exercised when interpreting TCR gene rearrangement results when using this as an indicator of clonality. We followed the clinical course of the patients with pseudo-spikes and found none to have developed a T cell malignancy in the intervening 1 year. More extensive follow-up may be warranted to determine the possible significance of such small peaks as a potential early detection marker.

We postulate that antigenic stimulation secondary to an underlying pathology in some of the cases may account for the pseudo-spikes. The immune system is stimulated in inflammatory conditions such as chronic pancreatitis.[14] Such antigenic stimulation may have resulted in the preferential proliferation of one or more clones, which manifested as a pseudo-spike. There are also studies showing cell-mediated immunity to be stimulated in various malignancies,[15,16] including specifically pancreatic cancer[17–18] (Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, Goemann M, Coleman J, Grochow L, Donehower RC, Lillimoe KD, O'Reilly S, Abrams RA, Pardoll DM, Cameron JL, Yeo CJ, manuscript submitted for publication). The majority of samples we analyzed came from patients who had either infection or malignancy, and this underlying process may have accounted for the pseudo-spikes observed. Studying the pattern of TCR-γ rearrangement in tissues harboring malignancy, chronic inflammation, or infection, particularly those in which cellular immunity is activated, and comparing this with the pattern of normal tissues from individuals without underlying infection or malignancy may give further insight into the origin of pseudo-spikes. Another theoretical explanation of the pseudo-spike could be the random preferential amplification of one or more rearranged TCR-γ gene in the early cycles during the PCR reaction that is perpetuated in the subsequent cycles, resulting in a pseudo-spike. However, we think this is unlikely, since the pseudo-spikes were reproducible in independent experiments.

Can one define a threshold below which peaks can be explained away as pseudo-spikes and above which the test is indicative of a neoplasm? We were reassured to note that the spikes observed from known T cell lymphoma samples were more definitively clonal than the pseudo-spikes (ie, they had higher h_1/h_2 ratios). The relative peak height was >2 in all 11 positive cases and >3 in 10/11 positive cases. These ratios are much higher

than what was observed with the benign samples, and may be a useful cutoff to distinguish between genuine monoclonal rearrangement and benign pseudo-spikes. Our data suggest that peaks with heights that are at least 3 times that of the normally distributed population is an appropriate threshold, indicative of a true clonal population in diagnostic samples. Such a cutoff correctly identified 10/11 known T cell lymphoma samples that were TCR-positive in our study. We have chosen 3 as a cutoff to be highly specific in diagnosing T cell clonality. This was a conservative choice based on our small sample size. Peaks that are less than 1.5 times that of the normally distributed population are seen in histologically benign samples, are likely to be insignificant, and may be disregarded as either noise or pseudo-spikes. Using these thresholds, all 53 histologically benign samples that were analyzed in our study would be correctly labeled as benign. Peaks that are from 1.5 to 3 times that of the normally distributed population should probably be designated indeterminate and warrant clinical correlation, further evaluation, and follow-up. More studies are required to determine the clinical relevance of these borderline peaks in the absence of other histological or molecular markers of T cell clonality. Additional studies with larger numbers of patients are warranted to confirm the appropriateness of the threshold values defined in this study.

Although assays for T cell clonality are used widely as adjuncts to diagnose malignancies originating from T cells, their use in the detection of minimal residual disease is not routine. This is because conventional PAGE lacks the sensitivity to detect small percentages of clones in a polyclonal background. Techniques such as Southern blotting or development of probes or primer sets unique to each patient's monoclonal rearranged TCR gene have been used for detection of minimal residual disease.[19,20] The latter analyses require sequencing the clonal spike and designing a tumor-specific primer. Once it is designed, the patient can be monitored for the level of the tumor, with the potential of determining the presence of minimal residual disease and diagnosing molecular relapse.[21–23] This may be particularly effective with the advent of real-time PCR technology that allows accurate and sensitive quantification.[24] Though such analyses may seem somewhat daunting in current clinical laboratory settings, they may ultimately prove efficacious and cost-beneficial, since therapy for molecular relapse (where only small numbers of cancer cells have recurred) may be substantially more effective than therapy after morphological relapse occurs.

CGE is a useful technique for the monitoring of minimal residual disease given its superior resolution and sensitivity. As CGE provides fairly precise sizing of the rearranged clone, this information should probably be included in the clinical report. This would be helpful when evaluating for minimal residual disease, where a rearranged clone of the same size can be specifically sought. In this situation, it might be appropriate to use thresholds lower than what we have defined for diagnostic specimens. It may also potentially provide useful information regarding the amount of monoclonal cells present with respect to polyclonal T cells, although more studies are required to define thresholds for such information. Since monitoring of disease relapse typically depends on evaluation of blood or bone marrow samples, it is reassuring to note a relatively low frequency and heights of pseudo-spikes in the normal blood samples we have analyzed, suggesting that the distinction between normal and malignancy can be more clearly defined in these tissue types. We read with great interest the recently published paper by Sprouse et al.[25] They describe an alternate method for approaching the same problem of quantifying in TCR-PCR assays the ratio of clonal to polyclonal components in samples containing both. Additional experiments may need to be performed to compare the two approaches directly.

CGE has better limit of detection and markedly better resolution than conventional PAGE, making it a valuable tool for diagnosing T cell neoplasms. However, one must account for the degree of noise and pseudo-spikes commonly observed in the histologically benign tissue samples. Once this is taken into account, CGE provides a markedly improved assay compared to conventional PAGE.

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