Interlocus V–J recombination measures genomic instability in agriculture workers at risk for lymphoid malignancies

(Stanley antigen receptor genes/leukemia/lymphoma)

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ABSTRACT V(D)J [variable–(diversity)–joining] rearrangements occur between, as well as within, immune receptor loci, resulting in the generation of hybrid antigen receptor genes and the formation of a variety of lymphocyte-specific chromosomal aberrations. Such hybrid genes occur at a low frequency in the peripheral blood lymphocytes (PBL) of normal individuals but show a markedly increased incidence in the PBL of individuals with the autosomal recessive disease ataxia-telangiectasia. In this manuscript we demonstrate that the frequency of hybrid antigen-receptor genes is 10- to 20-fold increased in the PBL of an occupational group, agriculture workers, with related environmental exposures. Both ataxia-telangiectasia patients and this population of agriculture workers are at increased risk for lymphoid malignancy. This result suggests that the measurement of hybrid antigen receptor genes in PBL may be a sensitive assay for a type of lymphocyte-specific genomic instability. As a corollary, this assay may identify populations at risk of developing common types of lymphoid malignancy.

In malignant lymphocytes there are data to suggest that the V(D)J [variable–(diversity)–joining] recombinase, which normally catalyzes recombination of DNA segments within each immunoglobulin and T-cell-receptor locus, may play a central role in the generation of genetic changes that dysregulate certain growth-affecting genes, such as c-MYC, BCL-2, or BCL-3. In addition to the aberrations found in malignant lymphocytes, the V(D)J recombinase may also generate chromosomal abnormalities seen in peripheral blood lymphocytes (PBL) from normal individuals. These abnormalities are due to V(D)J recombination between two antigen-receptor loci, and they result in potentially functional hybrid antigen-receptor genes (11–17). In patients with the inherited disease ataxia-telangiectasia (AT), a genetic instability syndrome associated with a high frequency of lymphoid malignancies (18), we have previously shown that the hybrid antigen-receptor gene formed by interlocus recombination between T-cell receptor γ (TCRγ) V segments and T-cell receptor β (TCRβ) J segments occurs in PBL at a frequency 50- to 100-fold higher than normal (17). This frequency is roughly the same as the increase in the risk for lymphoid malignancy of these individuals, so the frequency of hybrid antigen-receptor genes in the PBL of AT patients parallels their predisposition to lymphoid malignancy. There is also an increase in the frequency of the lymphocyte-specific cyogenetic abnormalities thought to be due to interlocus recombination in non-AT patients with non-Hodgkin lymphoma (19, 20), further suggesting a relationship between these “innocent” translocations and lymphoid malignancies.

Agriculture workers occupationally exposed to pesticides used in the production and storage of grain (such as phosphae, malathion, dichlorophenoxyacetic acid, chloropicrin, and captan) have a high frequency of cytogenetic abnormalities in their PBL (21), and we observed that the pattern of the abnormalities is reminiscent of the abnormalities seen in the PBL of AT patients. Furthermore, these agriculture workers are also at an increased risk of developing T- and B-lymphoid malignancies (22–26). Having established a PCR-based assay that identifies AT patients by their increased frequency of interlocus V(DJ) recombination, we wished to determine whether this same assay would also identify a subset of agriculture workers and, thus, provide a possible screening test for individuals with either genetic or acquired genomic instability and the related increased risk of developing lymphoid malignancies.

MATERIALS AND METHODS

Isolation of DNA. PBL were obtained by Ficoll/Hypaque (Pharmacia) density-gradient centrifugation (27) from heparinized blood. DNA was extracted as described (28).

PCR on Genomic DNA for Analysis of TCR Vγ-Jγβ Hybrids. Amplification reactions were done to assay rearrangements between TCRγ V and TCRβ Jp segments in a two-step nested PCR protocol as described (17). Briefly, 5′ primers were chosen that correspond to conserved sequences within the second exon of the Vγ1 V segments—a highly homologous family of V segments that represent 9 of the 14 known Vγ1 segments (29) (Fig. 1, Table 1). The 3′ primers were chosen in the intron 3′ of Jγ1p to allow amplification of rearrangements into any of the six functional Jγ1 segments (30). Each DNA sample underwent amplification in 75 μl of PCR reaction mix (17) with the outer 5′, Vγ primer and the outer 3′ Jγ1 primer. The mixture was heated to 95°C for 2.5 min, then underwent 25 cycles of 0.5 min at 95°C, 0.5 min at 50°C, and 6 min at 72°C, followed by 10 min at 72°C after the last cycle. Five microliters of the products of this first reaction underwent reamplification with the nested primers (Fig. 1, Table 1) in an identical thermal protocol. PCR products of different sizes ranging from ~230 base pairs (bp) to 2300 bp are generated depending on which Jp segment is used. Specific PCR products were demonstrated by agarose gel electrophoresis, Southern transfer to Nytran membranes, and hybridization to [α-32P]-labeled oligonucleotides internal to the amplification primers (Fig. 1, Table 1). Samples with known low and high frequencies of hybrid antigen-receptor genes were included in each titration experiment as controls of reaction sensitivity to allow comparisons between titration experiments. Amplification of a single-copy gene was done as a control to test the sample DNAs.

Study Population. Agriculture workers from rural Minnesota including workers involved in the production of grain,

Abbreviations: AT, ataxia-telangiectasia; TCRγ and TCRβ, T-cell receptor γ and β, respectively; PBL, peripheral blood lymphocytes; V(D)J, variable-(diversity)-joining.
dairy farmers, and organic farmers were selected for this study. This population had been the subject of an earlier study on pesticide-related genotoxicity (21). Control samples were collected from healthy individuals who did not work in agriculture or related occupations. All individuals studied were healthy, with no chronic illness, on no chronic medications, and without a history of prior or concurrent malignancy.

RESULTS

Measurement of Hybrid TCRγ–TCRB Antigen-Receptor Genes in PBL from Agriculture Workers. We assessed the occurrence of one type of hybrid antigen-receptor gene, formed by interlocus (V(D)J) recombination between TCRγ V segments and TCRβ J segments, generating the karyotypic abnormality (inv 7) (p13q35). The frequency of occurrence of this aberration was determined in the PBL from agriculture workers and a matched control population to determine whether the frequency of this entity could serve as an assay of genomic instability. Patients with AT were included as a positive control population because we had shown (17) that they have a marked increase in the frequency of this hybrid antigen-receptor gene in their PBL. Hybrid antigen-receptor genes formed by interlocus recombination between TCRγ V segments and TCRβ J segments in the genomic DNA from PBL of individuals in each group were assayed by a two-step PCR protocol with nested sets of oligonucleotide primers (Fig. 1; Table 1).

The presence of hybrid antigen-receptor genes formed by interlocus recombination between TCRγ V and TCRβ J segments was demonstrated in the PBL from all of the individuals in each sample population by this assay. The relative frequency of this hybrid antigen-receptor gene in each sample was determined by assaying dilutions of the DNA to assess the maximum dilution at which a hybrid gene could still be detected. Representative titrations for an agriculture worker, an AT patient, and a control individual are shown in Fig. 2. In all three samples, multiple bands can be seen representing the different possible rearrangements between the TCRγ V and the TCRβ J loci. This result is consistent with our previous data showing a polyclonal population of hybrid antigen-receptor genes in the lymphocytes from normal and AT individuals (17). The titration revealed that hybrid genes could be found at a greater dilution in the agriculture worker and the AT patient compared with the control (Fig. 2). As noted (17), larger PCR products were inefficiently amplified in the presence of competing smaller products and, therefore, an increased yield of large product is sometimes seen when the DNA is diluted to a point that the small product is no longer present. We also often noted skips in the titration curves at the furthest dilutions that most likely represent partitioning of a small number of targets into one tube. To demonstrate that the increased frequencies of hybrid genes observed was not from various abilities to amplify

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**Table 1. Sequence of oligonucleotides used as PCR primers or probes**

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Vα</td>
<td>TACATCCACTGTACCTACACCAAG</td>
</tr>
<tr>
<td>Vβ</td>
<td>CTGAGATTCGGTCTTACTTGGAATCCAG</td>
</tr>
<tr>
<td>Vγ</td>
<td>TCTGGGTGTTATTACTGTCACCTTG</td>
</tr>
<tr>
<td>Jβα</td>
<td>TTCCAGAACATGCTATGG</td>
</tr>
<tr>
<td>Jββ</td>
<td>CCGATTCCCGGATGCAAGA</td>
</tr>
<tr>
<td>Jβγc</td>
<td>CATACCCGTGCACTGGGAC</td>
</tr>
</tbody>
</table>

Locations of the oligonucleotides are indicated on Fig. 1. The oligonucleotides labeled a were used as primers in the first PCR reaction; those labeled b were used as internal primers in the second nested reaction; and those labeled c were used as probes. The Vγ oligonucleotides correspond to the coding strands. The Jβ oligonucleotides correspond to the inverted complement of the coding strands.

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**Fig. 1.** Idiogram of normal chromosome 7 and schematic representation of the TCRγ and TCRβ loci. Oligonucleotides used as PCR primers or probes are represented by arrows. Oligonucleotide sequences are described in Table 1.

**Fig. 2.** Southern blot analysis of amplified TCR Vγ-β hybrid genes from serially diluted genomic PBL DNA from a control individual, an agriculture worker, and an AT patient. Size markers are in bp. Amount of DNA (in μg) added per PCR reaction is shown at top. The blots were hybridized to the Jγc oligonucleotide probe (see Table 1). Hybridization to the Jγc oligonucleotide probe yielded identical results (data not shown). The three samples shown are the results from one experiment but are run on three separate gels, accounting for slight variations in the apparent product sizes.
targets from one sample or another, a control single-copy gene was amplified from dilutions of DNA samples from the three populations. There was no difference between samples from the three populations, and the control gene could be detected at a single-copy sensitivity from each.

Based on the results of the titrations, a relative frequency representing the number of hybrid genes per μg of DNA (or approximately 1.5 to 10³ PBL) was assigned to each sample. The relative frequency was defined as the reciprocal of the farthest dilution with a detectable PCR product. The values of these relative frequencies for the agriculture workers, the AT patients, and the control population are shown in Fig. 3. The mean relative frequency of hybrid genes in PBL for the agriculture workers (218 ± 83) and the AT patients (390 ± 127) was significantly greater than the control population (14 ± 4) (P < 0.02 and P < 0.01, respectively). The control and agriculture populations were pooled, and the median value was determined (dashed line, Fig. 3). Ten of 12 agriculture workers and all AT patients exceeded this median value, whereas only 1 of 10 control samples exceeded this cutoff. Thus, the fraction of agriculture workers and AT patients who had high frequencies of hybrid genes was significantly greater than the control population (P = 0.002 and P = 0.004, respectively).

Hybrid Antigen-Receptor Frequency and Use of Pesticides. The use of pesticides (including insecticides, herbicides, fumigants, and fungicides) is necessary for the large-scale production of grain and, as a result, most agriculture workers are exposed to one or more of these agents. Furthermore, the previously reported increase in cytogenetic abnormalities seen in the PBL of agriculture workers has been related to the use of pesticides (21). Although most agriculture workers studied in our sample had some exposure to one or more pesticides (such as phosphine, malathion, dichlorophenoxycetic acid, chloropicrin, and capitan), they could be divided into individuals with a low exposure and those with a high exposure, based on the amount of pesticide they applied and the frequency of use. The low-exposure group used small amounts of pesticides <10 days per season, whereas the high-use group used larger quantities of pesticides >10 times per season. As seen in Fig. 4, the mean relative frequency of hybrid antigen-receptor genes in the PBL from the high-use group was significantly greater than the mean frequency in the low-use group (P < 0.02). The high-use group also had a significantly greater mean relative frequency than the control population (P < 0.05), whereas the low-use group was not significantly different from controls. Because of the mixed exposure of most workers and the small numbers in this study, we cannot determine which agent(s) or combination of agents are related to the observed increase in hybrid gene frequency.

![Figure 3](image-url)  
**Fig. 3.** Frequency of TCR Vγ1-Jγ hybrid genes in the PBL DNA from controls, agriculture workers (AW), and AT patients. •, Relative frequencies (calculated as described in text) plotted for each individual in the population. – – –, Median value for pooled control and AW populations. Mean relative frequencies were compared by a two-tailed Student’s t test. The fractions of each population greater than the pooled median value were compared by a two-tailed Fisher exact test.

![Figure 4](image-url)  
**Fig. 4.** Relative hybrid antigen-receptor frequency and pesticide exposure. The agriculture workers were divided into low- or high-use groups based on their yearly pesticide use. Results represent the mean relative frequency ± SE for each group. Mean values were compared by a two-tailed Student’s t test.

![Figure 5](image-url)  
**Fig. 5.** Variation of hybrid gene frequency with seasonal pesticide use. Five agriculture workers who used large amounts of pesticides were studied at times during the growing season that were preexposure, during exposure, and postexposure. Results represent the relative frequency. Titrations to determine frequency were done at least in duplicate for each individual at each time point. The change in frequency for the paired samples was compared by a paired Wilcoxon signed-rank test. The mean frequencies at each sample time were compared to each other and to the controls (in Fig. 3) by a two-tailed Student’s t test.
Seasonal Variation of Hybrid Antigen-Receptor Gene Frequency. The use of pesticides by agriculture workers in rural Minnesota has a seasonal cycle with periods of high pesticide use during the growing season and periods of little or no use at other times. To further investigate the relationship of pesticide use to the frequency of hybrid antigen-receptor genes, five workers who applied large amounts of pesticides were studied at the height of pesticide use. Samples were available early in the growing season before pesticide use for four of these individuals and were available for four of these individuals as well from 3 to 6 mo later when they no longer were applying pesticides (Fig. 5). All of these individuals had applied pesticides for at least several years before this study.

In all workers studied, the relative frequency of hybrid antigen-receptor genes in their PBL was higher during their active exposure to pesticides than it was either before or after active pesticide use. Although the number of individuals sampled was small, the increase from preexposure to active exposure and the decrease from active exposure to postexposure approached statistical significance (P = 0.068 for both). All workers had relative frequencies above the pooled population median (shown in Fig. 3) during their exposure to pesticides. The mean frequency of hybrid genes in the exposed workers was significantly greater than the frequency in the controls (shown in Fig. 3) during their active pesticide use (P < 0.02) but was not significantly different from the controls in their pre- or postexposure samples. Only one of four workers had a frequency above the pooled population median at the first sampling time, suggesting that any increased frequency resulting from the previous year’s exposure was reversible. It may be of interest that the one abnormal individual at the first sampling time had the most dramatic increase in the frequency of hybrid genes with active pesticide exposure and used the greatest amount of pesticides of all those studied. No seasonal variation in the frequency of hybrid genes was seen in the samples from nonagriculture worker controls (data not shown). These data are consistent with previous cytogenetic observations that documented cytogenetic abnormalities in the PBL from workers exposed to pesticides that were transient and disappeared over time in the absence of further exposure (21).

DISCUSSION

In this paper, we describe an increased frequency of hybrid antigen-receptor genes in the PBL of agriculture workers and especially in those exposed to pesticides. These interlocus V(D)J recombinations create gross cytogenetic abnormalities, the breakpoints of which coincide with locations of the immunoglobulin and TCR genes. Such abnormalities have been described in normal PBL (8–10) and are increased in the PBL from agriculture workers exposed to pesticides (21) as well as in the PBL from AT patients (31, 32). Thus, there is an acquired genomic instability syndrome seen in agriculture workers and an inherited genomic instability syndrome seen in patients with AT; affected individuals can be identified by the increased frequency of a particular hybrid antigen-receptor gene in their PBL. This PCR assay of a hybrid antigen-receptor gene is, thus, a marker of a kind of genomic instability in these populations.

The specific hybrid antigen receptor gene formed by interlocus V(D)J recombination between TCRγ and TCRβ has no defined transforming activity and thus likely results in an “innocent” translocation. The V(D)J recombinase system is also known to be involved in the generation of translocations in lymphoid malignancies that result in the dysregulation of genes that affect cell growth and differentiation (2–7). These translocations are often involved in loci that are not antigen-receptor genes and result in pseudo-V-J recombinations. Such translocations can also involve two nonantigen receptor genes that have the appropriate recombination signals in their DNA sequence (33). The basic mechanism for interlocus recombination, therefore, appears similar whether it is generating an “innocent” hybrid antigen-receptor gene or generating a “malignant” translocation. Thus, the measurement of V(D)J recombinase-mediated chromosomal aberrations is not only a measure of genomic instability, but it is also a measure of the underlying mechanism capable of generating transforming translocations in lymphoid cells. The leukemias and lymphomas for which AT patients and agriculture workers are at risk, in general, those in which the V(D)J recombinase has been implicated as contributing to transformation (18, 22–26), such as the t(14;18) of follicular lymphomas, which involves site-specific recombination between the immunoglobulin heavy chain and BCL-2 (34) and the t(11;14) seen in intermediate and high-grade lymphomas, which involves rearrangement between the immunoglobulin heavy chain and BCL-1 (35).

The basis for the increased frequency of hybrid genes in the PBL of agriculture workers and AT patients is unknown. (i) One explanation could be a perturbation of chromatin structure that would result in increased accessibility of the T-cell receptor loci and, thus, a higher frequency of interlocus V(D)J rearrangement (for review of antigen-receptor accessibility, see ref. 36). (ii) Alternatively, abnormal catalytic properties or level of activity of the V(D)J recombinase may account for the increased frequency. (iii) A third possibility is abnormal clearance or trafficking of the lymphocytes that carry the hybrid genes resulting in a greater number in the PBL than seen in normal individuals. Although the underlying mechanism that causes the increased frequency of hybrid genes in agriculture workers and AT may, in fact, be the same, there is a fundamental difference in the etiology. AT is a genetic disease with a continuously expressed phenotype, whereas in the agriculture workers some exogenous agent seems to induce a transient, acquired abnormality. The incidence of lymphoid malignancies is increased >100-fold in AT patients (18) but is only increased 5- to 10-fold in agriculture workers (22–26). This relationship is consistent with the continuous defect seen in AT patients and the transient defect seen in agriculture workers.

In summary, in both agriculture workers exposed to pesticides and AT patients there is an increased frequency of lymphoid malignancies (18, 22–26). We have shown that both groups demonstrate an elevated level of genomic instability as measured by an increased frequency of hybrid antigen-receptor genes in their PBL. Together, these data suggest a correlation between an increased frequency of hybrid antigen-receptor genes and the predisposition to develop a lymphoid malignancy. The measurement of the frequency of hybrid antigen-receptor genes in the PBL may thus identify populations at increased risk for the development of certain lymphoid malignancies.

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