Hybrid T Cell Receptor Genes Formed by Interlocus Recombination in Normal and Ataxia-telangiectasia Lymphocytes

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Summary

In this paper, using polymerase chain reaction (PCR), we demonstrated the occurrence of hybrid genes formed by interlocus recombination between T cell receptor γ (TCR-γ) variable (V) regions and TCR-β joining (J) regions in the peripheral blood lymphocytes (PBL) from normal individuals and patients with ataxia-telangiectasia (AT). Sequence analysis of the PCR-derived hybrid genes confirmed that site-specific Vγ-Jβ recombination had occurred and showed that 10 of 23 genomic hybrid genes maintained a correct open reading frame. By dilution analysis, the frequency of these hybrid genes was 8 ± $1/10^5$ cells in normal PBL and 587 ± $195/10^5$ cells in AT PBL. These frequencies and the ~70-fold difference between the normal and AT samples are consistent with previous cytogenetic data examining the occurrence of an inversion of chromosome 7 in normal and AT PBL.

We also demonstrated expression of these hybrid genes by PCR analysis of first-strand cDNA prepared from both normal and AT PBL. Sequence analysis of the PCR-amplified transcripts showed that, in contrast to the genomic hybrid genes, 19 of 22 expressed genes maintained a correct open reading frame at the V-J junction and correctly spliced the hybrid V-J exon to a TCR-β constant region, thus allowing translation into a potentially functional hybrid TCR protein. Another type of hybrid TCR transcript was found in a which a rearranged TCR-γ V-J exon was correctly spliced to a TCR-β constant region. This form of hybrid gene may be formed by *trans*-splicing.

These hybrid TCR genes may serve to increase the repertoire of the immune response. In addition, studies of their mechanism of formation and its misregulation in AT may provide insight into the nature of the chromosomal instability syndrome associated with AT. The mechanism underlying hybrid gene formation may be analogous to the mechanism underlying rearrangements between putative growth-affecting genes and the antigen receptor loci, which are associated with AT lymphocyte clones and lymphoid malignancies.

We previously described recombination that occurs between loci of the Ig and TCR genes. We and others (1–3) first demonstrated such an interlocus recombination by molecular characterization of an inversion of chromosome 14 in a T cell lymphoma cell line. The inversion resulted from site-specific recombination between an Ig H chain V region and a TCR-α J region. This recombination resulted in an in-frame, transcribed hybrid gene that could be translated into a functional hybrid antigen receptor. We described a second example of a hybrid Ig-TCR gene in the leukemic cells from a patient with acute lymphocytic leukemia (4). Again, an in-frame hybrid gene was formed that was transcribed and could potentially be translated.

These two hybrid genes came from frankly malignant lymphocytes. However, the contribution of these hybrid genes to the growth of the cells in which they were found is unclear since morphologically identical chromosomal abnormalities occur in PBL from normal individuals (5, 6). In fact, a variety of inversions and translocations involving two sites on both chromosome 7 and 14 can be seen in the metaphase chromosomes from normal lymphocytes (5–7). The breakpoints of these abnormalities coincide with the locations of the TCR and Ig H chain loci (8). We wanted to study the molecular structure of such cytogenetic abnormalities in nonmalignant lymphocytes to understand their relationship to those already characterized, and to determine what if any role these hybrid genes might have in cell growth and differentiation.

Our investigation began with an analysis of a nonmalignant cell line carrying one such abnormality, an inversion of chromosome 7 [inv (7)], derived from a patient with ataxia-

telangiectasia (AT).[1] AT is a disease characterized by progressive cerebellar degeneration, oculocutaneous telangiectasia, variable immunodeficiency, radiation sensitivity, chromosomal instability, and a predisposition to lymphoid malignancies (9, 10). Between 1 and 5% of the peripheral T lymphocyte metaphases in this patient, from whom the cell line was derived, carried an inv(7), but there was no evidence of a lymphoid malignancy (11). We showed that the inv(7) in the cell line resulted from site-specific recombination between a V region of TCR-γ and a J region of TCR-β (12). Again, the hybrid gene was in-frame, transcribed, and could potentially be translated into a hybrid antigen receptor. This hybrid gene could also be found in the PBL from the patient.

In the next phase of this work, presented here, we attempt to generalize this finding by assessing the structure, frequency, and expression of hybrid TCR genes, formed by interlocus recombination between TCR-γ and TCR-β, in the lymphocytes from normal individuals, AT patients, and family members of AT patients (none of whom had leukemia or lymphoma). Through this investigation, we have not only gained insight into the mechanism of formation of these hybrid genes in normal, AT, and malignant lymphocytes, but may have begun to gain insight into the basis of the chromosomal instability syndrome associated with AT.

Materials and Methods

Isolation of DNA and RNA. Human PBMC were obtained by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation (13) from heparinized blood from patients with a clinically established diagnosis of AT (14) or from healthy normal subjects. The AT patients were between 15 and 40 yr old, and the normal individuals were between 20 and 40 yr old. DNA and RNA were extracted as previously described (15, 16). Negative control DNA and RNA were prepared as above from the T cell line SUP-T1, which is a cell line without an inv(7) chromosomal abnormality (17).

Oligonucleotides. Oligonucleotides were synthesized on a DNA synthesizer (380B; Applied Biosystems, Foster City, CA) and used without further purification.

PCR on Genomic DNA for Analysis of Vγ-Jβ Hybrids. Separate amplification reactions were performed to assay rearrangements between Vγ and Jβ1 segments or Vγ and Jβ2 segments. A two-step nested PCR protocol was performed by a modification of the method of Saiki et al. (18). In the first step, DNA (1 μg or an appropriate dilution) was diluted into a 75-μl solution containing 200 μM dGTP, 200 μM dATP, 200 μM dTTP, 200 μM dCTP (Pharmacia Fine Chemicals), 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 2.5 U Taq polymerase (Cetus Corp., Emeryville, CA), and 10% (vol/vol) DMSO (Sigma Chemical Co., St. Louis, MO), and 0.1 μg of each of the primers to amplify Vγ-Jβ1 rearrangements (Vγa and Jβ1a) (Fig. 1; Table 1), or 0.1 μg of each of the primers to amplify Vγ-Jβ2 rearrangements (Vγa and Jβ2a) (Fig. 1; Table 1). DMSO was added to the PCR reactions because it was found to increase the yield of larger products when amplified simultaneously with smaller products. The sample was overlayered with light mineral oil (Fisher Scientific Co., Pittsburgh, PA). To amplify the DNA, 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. In the second step, 5 μl of the first amplification reaction was diluted into 70 μl of an identical solution containing either 0.5 μg of each of the nested primers to amplify the Vγ-Jβ1 rearrangements (Vγb and Jβ1b) or 0.5 μg of each of the nested primers to amplify the Vγ-Jβ2 rearrangement (Vγb and Jβ2b) (Fig. 1; Table 1). An identical thermal protocol was then performed. A consistent finding was that at high DNA concentrations, when multiple competing targets were present, short products amplified more efficiently than larger products. At lower DNA concentrations, when the larger product titrated further than the smaller product, the amplification of the larger product increased, despite fewer templates initially (e.g., Fig. 3). Longer exposures of the autoradiograph demonstrated the presence of the larger amplified products as faint bands when the shorter products were present.

Analysis of RNA by PCR (Reverse Transcriptase [RT]-PCR). RT-PCR was performed by a modification of a previously described method (19). First-strand cDNA was synthesized in a 20-μl reaction containing 5.0 μg of unfractionated total RNA, 1 mM dGTP, 1 mM dATP, 1 mM dTTP, 1 mM dCTP, 75 mM KC1, 50 mM Tris(pH 8.3), 10 mM DTT, 3 mM $MgCl_2$ (BRL 5× buffer; Bethesda Research Laboratories, Gaithersburg, MD), 200 U of M-MLV RT (Bethesda Research Laboratories), and 0.4 μg of the Cβa oligonucleotide primer (Fig. 1; Table 1). Equal amounts of RNA, measured by OD a 260 nM and by visual assessment on an ethidium bromide–stained agarose gel, were used for each reaction. The reaction mix was incubated at 37°C for 30 min, then at 95°C for 5 min (to inactivate the enzyme) and stored at −20°C. Amplification of hybrids was performed by diluting 10 μl of first-strand cDNA into 90 μl containing 200 μM dATP, 200 μM dTTP, 200 μM dCTP, 50 mM KCL, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 2.5 U Taq polymerase, and 0.5 μg of Vγb and Cβb oligonucleotides (Fig. 1; Table 1). The sample was heated to 95°C for 2.5 min, then underwent 50 cycles of 0.5 min at 95°C, 0.5 min at 55°C, and 2 min at 72°C, followed by 10 min at 72°C after the last cycle. Control amplification of intact TCR-β mRNA was performed by diluting 2 μl of first-strand cDNA to 10 μl with water and then diluting this into 90 μl of PCR mixture as described above, except containing 0.5 μg of the Vβa oligonucleotide (Fig. 1; Table 1) instead of the Vγb oligonucleotide. The same thermal profile was performed.

Analysis of PCR Products. The amplified samples were extracted with $CHCl_3$ to remove the mineral oil, and one half of each sample was analyzed by electrophoresis on a 1.5% agarose gel, Southern transfer (20) to a Nytran membrane (Schleicher & Schuell, Inc., Keene, NH), and hybridization to appropriate oligonucleotide probes internal to the amplification primers (Fig. 1; Table 1). Alkaline transfer (21) was found to be inefficient at transferring fragments <500 bp, and so Southern transfer was used. Oligonucleotides used as probes were end labeled to a sp act of 10^7–10^8 cpm/μg DNA with α-[^{32}p]dCTP (Amersham Corp., Arlington Heights, IL) by terminal deoxynucleotidyl transferase (Bethesda Research Laboratories), as described (22). The Nytran membrane was prehybridized for 1 h at 50°C in hybridization buffer (6× SSC, 10× Denhardt's, and 50 μg/ml sheared herring sperm DNA). Labeled oligonucleotide in 1 μg denatured sheared herring sperm DNA and fresh hybridization buffer (at 10^6 cpm/ml) was added to the Nytran membrane and incubated overnight at 50°C. Membranes were washed three times at room temperature with 6× SSC, 0.1% (wt/vol) SDS, followed by high temperature washes with 6× SSC, 0.1% (wt/vol) SDS at the calculated melting temperature (Tm)

[1] *Abbreviations used in this paper:* AT, ataxia-telangiectasia; inv(7), inversion of chromosome 7; RT, reverse transcriptase.

for each oligonucleotide probe minus 5°C. Autoradiography was performed at −80°C with an intensifying screen and XRP film (Kodak, Rochester, NY) for 1–16 h.

Sequence Analysis of PCR Products. Amplified material (from genomic or RT-PCR) was digested with EcoRI and BamHI restriction endonucleases (Bethesda Research Laboratories) and ligated into a pGEM-7Zf(+) plasmid vector (Promega Biotec, Madison, WI). Transfected bacterial cells were screened for recombinants using oligonucleotides internal to the amplifying primers (Fig. 1; Table 1). Plasmid DNA was prepared by alkaline lysis (23) and subsequent ribonuclease A (Sigma Chemical Co.) treatment. Plasmid DNA was sequenced using the dideoxy chain termination technique (24) and Sequenase version 2.0 (United States Biochemical Corp., Cleveland, OH). Sequences were compared with published sequences for TCR-β (25) or TCR-γ (26, 27).

Statistics. Genomic titration results for normal and AT samples were compared by a two-tailed student's *t* test. The fraction of open reading frames in genomic and cDNA clones was compared by a χ^2 test.

Results

The Occurrence of TCR-γ V-TCR-βJ Hybrids in Lymphocyte DNA from Normal Individuals and AT Patients. With the hybrid TCR gene found in the AT cell line as a precedent, we then undertook an analysis of such hybrid genes in the peripheral blood T cell population of normal individuals and five other AT patients. Interlocus recombination between TCR-γ V regions and TCR-β J regions in the genomic DNA from PBL of AT patients and normal individuals was assayed by a two-step PCR reaction with nested sets of oligonucleotide primers. 5′ oligonucleotides were chosen that correspond to conserved sequences within the second exon of the VγI variable regions (Fig. 1; Table 1), a highly homologous family of variable regions that represent 9 of the 14 known Vγ variable regions (26). There are two distinct clusters of J segments within the TCR-β locus (25). One, Jβ1, consists of six J segments, and the other, Jβ2, consists of seven J segments. Two separate sets of 3′ oligonucleotides were chosen (one set in the intron 3′ of Jβ1.6 and the other set in the intron 3′ of Jβ2.7) to allow amplification of rearrangements into either the Jβ1 or Jβ2 locus, respectively (Fig. 1; Table 1). Each DNA sample underwent amplification with the 5′ Vγ oligonucleotides and the 3′ Jβ1 or 3′ Jβ2 oligonucleotides in two separate reactions. PCR products of different sizes are generated in this system depending on which Jβ segment is utilized from either the Jβ1 or Jβ2 clusters. The predicted sizes would range between ~230 and ~2,350 bp for the six Jβ1 segments, and would range between ~250 and ~1,350 bp for the seven Jβ2 segments. Specific PCR products were demonstrated by agarose gel electrophoresis, Southern transfer to Nytran membranes, and hybridization to α-[^{32}P]-labeled oligonucleotides internal to the amplification primers (Fig. 1; Table 1).

Vγ-Jβ interlocus recombination utilizing both Jβ1 and Jβ2 regions was demonstrated in the genomic DNA from PBL of AT patients and normal individuals (Fig. 2). Multiple PCR products of different sizes were seen in the five AT patients, while a more limited number of PCR products of different sizes were seen in the three normal individuals (Fig. 2). In addition, the signal intensity of the PCR products from the AT patients was greater than the intensity of the PCR products in the normal individuals. An obligate heterozygote for AT and an unaffected sibling of an AT patient gave results similar to the normal individuals (Fig. 2). No specific PCR products were seen in DNA from SUP-T1, a T cell line without cytogenetic evidence of an inversion of chromosome 7 (data not shown). Longer exposure of the autoradiographs demonstrated faint bands corresponding to the larger predicted PCR products in all of the AT samples and some of the normal samples for the Jβ1 locus (data not shown). The observed PCR products spanned the size range predicted for each locus, and hybridized to oligonucleotide probes internal to both the Jβ oligonucleotide primers (Fig. 2) and the Vγ oligonucleotide primers (data not shown).

The PCR-amplified hybrid TCR genes were cloned into a pGEM-7Zf(+) plasmid vector, and multiple clones from an AT patient and a normal individual were sequenced (Table

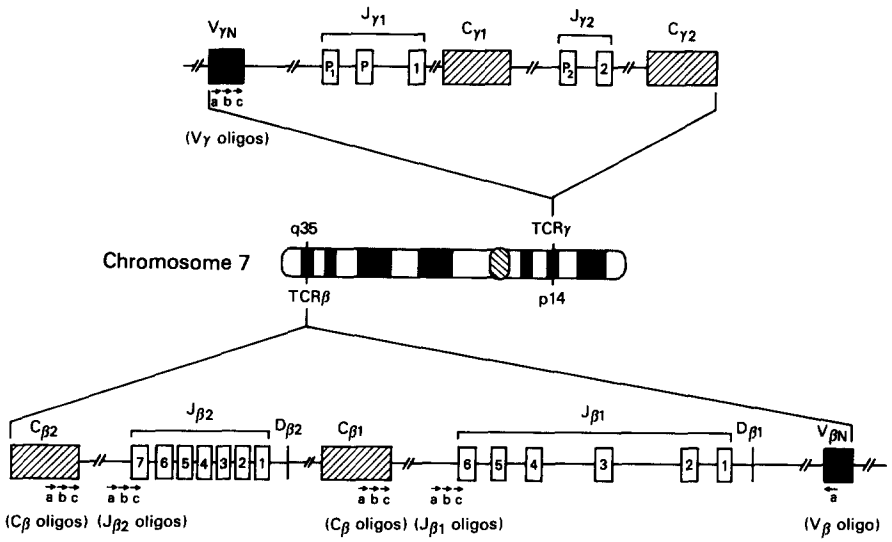

Figure 1. Idiogram of normal chromosome 7 and schematic representation of the TCR-γ and TCR-β loci. Oligonucleotides used as PCR primers or probes are represented by arrows. Oligonucleotide sequences and their use are described in Table 1.

Table 1. *Sequence of Oligonucleotides Used as PCR Primers or Probes*

Oligonucleotide	Sequence
Vγa	TACATCCACTGGTACCTACACCAG
Vγb	CTAGAATTCCAGGGTTGTGTTGGAATCAGGA
Vγc	TCTGGGGTCTATTACTGTGCCACCTGG
Vβa	TCTGTGTACTGGTACCAACAG
Jβ1a	TTCCCAGCAACTGATCATTG
Jβ1b	CCAGGATCCCCCGAGTCAAGA
Jβ1c	CATACCTGTCACAGTGAGCC
Jβ2a	TTGCAGAGCTGACCC
Jβ2b	AGCGGATCCAGCTCCGGTCCA
Jβ2c	CTCACCTGTGACCGTGAGCC
Cβa	CAGCTCAGCTCCACGTGGTC
Cβb	GAAGGATCCTGTGGCCAGGCACACCAGTGT
Cβc	TGTGGGAGATCTCTGCTTCTGAT

The location of the oligonucleotides is indicated in Fig. 1. The oligonucleotides labeled *a* were used as primers in the first PCR reaction. The oligonucleotides labeled *b* were used as internal primers in the second "nested" reaction. The oligonucleotides labeled *c* were used as probes. The Vγ and Vβ oligonucleotides correspond to the coding strand. The Vγb primer has an EcoR1 site introduced to facilitate subsequent cloning of PCR products. The Jβ1, Jβ2, and Cβ oligonucleotides correspond to the inverted complement of the respective coding strands. The Jβ1b, Jβ2b, and Cβb primers have a BamH1 site introduced to facilitate subsequent cloning of PCR products.

2). This analysis revealed that the interlocus recombination occurred in a site-specific fashion analogous to the intralocus recombination normally described for Ig or TCR genes (28). The Vγ regions showed variability in the exact nucleotide at which the recombination occurred, and were followed by a variable number of nucleotides that could not be assigned to either the Vγ or Jβ locus (so-called N-region nucleotides). The Jβ regions also showed variability in the exact nucleotide at which recombination occurred. There were no recognizable Dβ regions. All but one of the Vγ regions utilized could be assigned to either Vγ 1.2, 1.3, 1.4, 1.5, or 1.8, all of which are known functional Vγ genes (26). One clone from the normal PBL (clone 6) utilized Vγ 1.7, which is a nonfunctional Vγ region due to a deletion within its coding sequence (26). Within the Vγ and Jβ sequences there were occasionally base changes compared with reported sequences. These might represent either polymorphisms or PCR-generated artifacts. An open reading frame that would allow translation of a hybrid Vγ-Jβ region was present in 8 of 16 AT genomic clones and two of seven normal genomic clones.

The greater number of different sized PCR products amplified from each locus in the AT PBL DNA compared with normal PBL DNA (Fig. 2) suggested a greater heterogeneity of hybrid genes in AT. This was confirmed by the sequence analysis (Table 2), since no duplicate clones were found in the 16 clones fully sequenced. This observation was extended further by comparing partial sequences of 10 additional clones, which again demonstrated no duplicates. The same results were obtained in another AT patient. Similarly, the more restricted heterogeneity of hybrid genes in PBL from a normal individual (Fig. 2) was confirmed by sequence analysis (Table 2), since duplicate clones were often found, and only nine unique clones were found in the 18 clones sequenced. Similar results were obtained in a second normal individual.

One clone (clone 7) derived from a normal individual showed rearrangement between a Vγ region and the intron immediately 3′ of Jβ2.7.

The Frequency of Hybrid Gene Formation. The frequency of hybrid TCR genes in the PBL of five AT patients and five apparently normal individuals was determined by PCR amplification of serial dilutions of the DNA samples. Separate titrations were performed on the DNA from each diluted sample using primers for either the Jβ1 cluster or the Jβ2 cluster. A representative titration for Vγ-Jβ1 or Vγ-Jβ2 rearrangement is shown for one AT patient and one normal individual (Fig. 3). It was consistently possible to dilute the DNA derived from AT patients 1–2 logs further (down to 10^{-2} to 10^{-3} μg) than normal individuals and still detect Vγ-Jβ hybrids. The frequency of hybrid genes per 10^5 cells was calculated from the farthest dilution with a detectable PCR product for both loci of each sample, assuming single-copy sensitivity for the PCR reaction and 1.5×10^5 cell equivalents per microgram of DNA. The frequencies for the two Jβ loci were added to give the frequency of hybrid genes. The AT patients had 587 ± 195 Vγ-Jβ recombinants/10^5 cells (range, 133–1,000), while the normal individuals had 8 ± 1 recombinants/10^5 cells (range, 4–10), for an ~70-fold difference that was statistically significant ($p < 0.02$). There was no preferential utilization of the Jβ1 or Jβ2 loci for either normal or AT samples.

Different sized PCR products (representing utilization of different Jβ sequences) titrated out to different dilutions, indicating that the frequency of some Vγ-Jβ recombinants was greater than others. The predominant Jβ recombination varied from patient to patient.

Identification of TCR-γV-TCR-βJ Hybrids mRNA in Normal and AT Lymphocytes. We assayed for expression of mRNA transcripts from the hybrid TCR genes that had occurred in normal and AT PBL. Expression of mRNA transcripts from the Vγ-Jβ recombinants was demonstrated by the use of RT-PCR (Fig. 4). A specific PCR product of the predicted size (~300 bp), which hybridized to a labeled oligonucleotide internal to the Vγ primer (Fig. 4*A*) and to a labeled oligonucleotide internal to the Cβ primer (Fig. 4 *B*), was seen in AT patients and normal individuals. As a negative control, no specific PCR product was seen in the SUP-T1 cell line (data not shown). The intensity of the signal from the AT samples was much greater than the intensity of the signal from the normal samples, consistent with a greater abundance of mRNA transcripts in the AT samples. Dilution analysis of the first-strand cDNA prepared from RNA of AT and normal PBL revealed ~10–100 times more transcripts from the AT samples. Amplification of rearranged

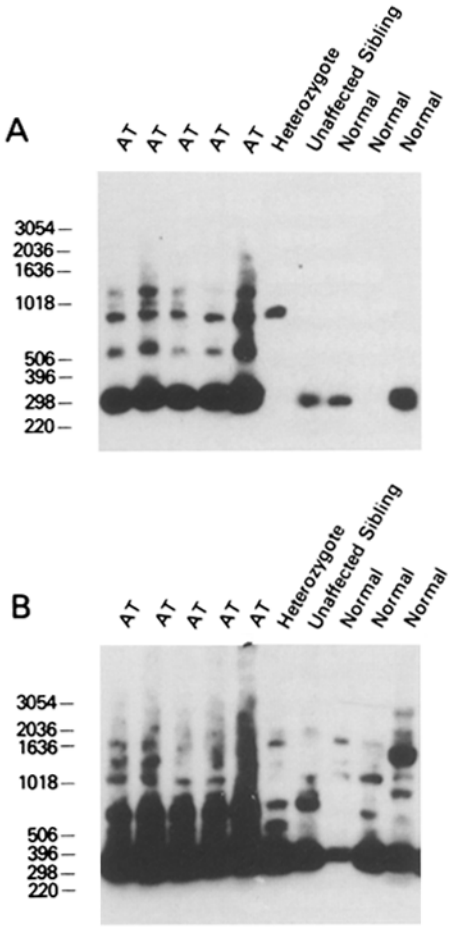

Figure 2. Southern Analysis of amplified Vγ-Jβ hybrid genes from genomic PBL DNA. Amplified products of Vγ-Jβ1 PCR reaction were hybridized to Jβ1c oligonucleotide probe (*A*). Amplified products of Vγ-Jβ2 PCR reaction were hybridized to Jβ2c oligonucleotide probe (*B*). Size markers are in base pairs. Identical results were obtained when both blots were stripped and rehybridized to a Vγ oligonucleotide probe (data not shown). Lanes *1–5*, AT; lane *6*, AT heterozygote; lane 7, unaffected sibling of AT patients; lane *8–10*, normal (NL) individuals.

TCR-β (Vβ-Jβ-Cβ) resulted in a product of equal signal intensity from all of the samples (Fig. 4 *C*), demonstrating that the differences seen above (Fig. 4, *A* and *B*) were not due to qualitative differences in the RNA.

The cDNA products from one AT patient and one normal individual were cloned into a pGEM-7Zf(+) plasmid vector, and multiple clones were sequenced (Table 3). Sequence analysis confirmed that these clones were Vγ-Jβ recombinants, except for four unusual clones discussed below. 9 of 13 AT cDNA clones and 7 of 11 normal cDNA clones utilized the same Vγ region (Vγ 1.4). This preferential Vγ utilization had not been seen in the genomic clones (Table 2). This analysis also demonstrated correct RNA splicing between the V-J exons and Cβ exons in all of the clones sequenced. The AT patient was found to have one predominant cDNA clone comprising 6 of 18 independent clones sequenced. No other AT clone was found in duplicate. The normal clones more frequently had duplicates, and only 11 different clones were found among the 25 clones sequenced. Open reading frames were present in 11 of 12 different cDNA clones from the AT patient and 8 of 10 different cDNA clones from the normal individual. This predominance of cDNA clones maintaining a correct open reading frame was significantly greater than the results for the genomic clones ($p < 0.005$).

Two cDNA clones from the normal individual (clones 3 and 9) showed recombination between a Vγ and Jγ segment, but this product was then spliced to a Cβ1 exon. These clones maintained a correct open reading frame at the V-J junction and had apparently correct RNA splicing between the Vγ-Jγ exon and the Cβ1 exon. Two other cDNA clones, one from the AT patient (clone 3) and one from the normal individual (clone 7), had no J region. Instead, a Vγ region followed by several N-region nucleotides was joined directly to the first base of the Cβ2 exon. Both clones maintained an open reading frame at the Vγ-N-Cβ junction.

Discussion

Utilizing a sensitive PCR technique, we have demonstrated site-specific interlocus recombination between TCR-γ V regions and TCR-β J regions in the PBL from normal individuals and AT patients. The sequence analysis revealed V-J junctions that were analogous to those normally formed by recombination within a given locus (28, 29). This suggests utilization of the normal recombination signals common to both of these loci by the recombinase(s) and further implies that both loci were simultaneously accessible to the recombinase. In the ontogeny of the TCR genes, Vγ-Jγ rearrangements are thought to occur simultaneously with Dβ-Jβ rearrangements, but are thought to precede Vβ-DβJβ rearrangement (30). Thus, both loci are at least partly accessible to the recombinase machinery simultaneously. Interestingly, none of the Vγ-Jβ recombinants had recognizable Dβ sequences, suggesting that the recombination occurred before the initial Dβ-Jβ recombination, or that once Dβ-Jβ recombination occurs interlocus recombination is precluded.

Interlocus recombination between TCR-γ V regions and TCR-β J regions should generate an inversion of chromosome 7. Such an inv(7) is one of the recurrent T cell–specific cytogenetic abnormalities that has been described in normal and AT PBL (5–7, 31, 32). Our work demonstrates an ~70-fold increase in the frequency of Vγ-Jβ recombination in AT PBL compared with normal PBL. This result is consistent with previous cytogenetic analyses demonstrating an increased frequency of the inv(7) in AT PBL compared with normal PBL (31, 32). Our data are consistent with an increased frequency of independent rearrangements resulting in a polyclonal population of lymphocytes each bearing an inv(7) in AT.

The increased frequency of hybrid antigen receptor formation in AT lymphocytes could result from abnormal catalytic function of the recombinase in AT. However, the V-J junctions formed in the hybrid genes are structurally normal and are formed in accordance with the rules of recombination derived from studies of normal V-J recombination (28, 29). This suggests normal catalytic properties of the recom-

Table 2. *Sequences of V-J Junctions of Genomic Clones*

Clones	V	N	J	Open reading frame
AT				
1	(Vγ 1.2) **GCCACCTGGGACGGG**	G	GAA**AAA** (Jβ 1.4)	–
2	(Vγ 1.4) **GCCACCTGGGATGG**	ACCAAGCAATG	**GCCC** (Jβ 1.5)	–
3	(Vγ 1.5) **GCCACCTGGGACAGG**	CCGGTATA	AAT**TCA** (Jβ 1.6)	–
4	(Vγ 1.4) **GCCACCTGGGATG**	AGAATAGAGGTGG	TCAG**CCC** (Jβ 1.5)	+
5	(Vγ 1.4) **GCCACCTGGGATGGG**	CCC	TCCTATAAT**TCA** (Jβ 1.6)	+
6	(Vγ 1.4) **GCCACCTGGG**	CCTCCCCCC	TCCTATAAT**TCA** (Jβ 1.6)	–
7	(Vγ 1.3) **GCCACCTGGGAC**	TCTGTATAAGG	GCAATCAG**CCC** (Jβ 1.5)	–
8	(Vγ 1.2) **GCCACCTGGG**	TAA	CTACGAG**CAG** (Jβ 2.7)	–
9	(Vγ 1.3) **GCCACCTGGGAC**	GAC	TCCTACGAG**CAG** (Jβ 2.7)	+
10	(Vγ 1.2) **GCCACCTGGGACG**	AGATC	TCCTACGAG**CAG** (Jβ 2.7)	+
11	(Vγ 1.2) **GCCACCTGGGACGGG**	CAACGTCCACGAC	**CAG** (Jβ 2.7)	–
12	(Vγ 1.3) **GCCACCTGGG**	TCCGGCCTGGGAGT	TACGAG**CAG** (Jβ 2.7)	+
13	(Vγ 1.5) **GCCACCTGGGACA**	CCCCATAGACCCC	CTGGGGCC**AAC** (Jβ 2.6)	–
14	(Vγ 1.4) **GCCACCTGGGATG**	AC	TCTGGGGCC**AAC** (Jβ 2.6)	+
15	(Vγ 1.8) **GCC**	CCGCGCGAGTTC	CAAGAGACC**CAG** (Jβ 2.5)	+
16	(Vγ 1.5) **GCCACCTGG**	ACGGGAGGGG	GCACAGAT**ACG** (Jβ 2.3)	+
Normal				
1	(Vγ 1.4) **GCCACCTGGGATGGG**	CGTATCGATACTCCCCCTA	ATAAT**TCA** (Jβ 1.6)	+
2*	(Vγ 1.3) **GCCACCTGGGACAGG**	TTCC	TAAT**TCA** (Jβ 1.6)	–
3	(Vγ 1.8) **GCCACCTGGGA**	GAGTTC	AATGAA**AAA** (Jβ 1.4)	–
4*	(Vγ 1.8) **GCCACCTGGGA**	GAAGGGGGT	AGCAATCAG**CCC** (Jβ 1.5)	–
5	(Vγ 1.3) **GCCACCTGGGACAGG**	TAGCGGGAACGG	TACGAG**CAG** (Jβ 2.7)	–
6*	(Vγ 1.7) **GCCACCTGGGACAGG**	CCCCAGCCGGGCG	CCGGG**GAG** (Jβ 2.2)	(NA)‡
7*	(Vγ 1.8) **GCCACCTGGGA**	ATG	CTTCCAGCC**CCT** (3′Jβ 2.7)	(NA)§
8	(Vγ 1.4) **GCCACCTGGCATG**	T	CAAAAAC**ATT** (Jβ 2.4)	+
9	(Vγ 1.8) **GCCACC**	GAGTAAGCGGGGGGCCAAGGG	AGAT**ACG** (Jβ 2.3)	–

Vγ variable regions (V) were assigned based on at least 90 bases of sequence. Sequence is shown beginning at the fifth codon (bold nucleotides) from the 3′ end of the germline Vγ I genes. The Jβ regions (J) were assigned based on their entire coding sequence. Sequence is shown ending with the fourth complete codon (bold nucleotides) from the 5′ end of the germline Jβ genes. N-region nucleotides (N) represent those nucleotides that could not be assigned to either Vγ or Jβ segments. Open reading frames were considered positive if correct reading frame was maintained with respect to both Vγ and Jβ.

* Multiple identical clones were found.

‡ This clone utilized Vγ 1.7, a nonfunctional Vγ I gene due to a deletion in the coding region. Therefore, determination of open reading frame is not applicable (NA).

§ This clone rearranges into the intron 3′ of Jβ 2.7. Therefore, determination of open reading frame is not applicable (NA).

binase. It is possible that the recombinase, while catalytically normal, is over expressed or hyperactive. Another explanation, consistent with the data, is abnormal accessibility of the different antigen receptor loci to the recombinase. Such a general defect of accessibility might tie together the varied phenotypic findings of patients with AT.

Expression of mRNA transcripts from interlocus Vγ-Jβ recombinants was observed in both AT and normal PBL. There was a greater abundance of mRNA transcripts in the AT PBL consistent with the increased occurrence of such recombinants in the genomic DNA of AT PBL. 11 of 12 AT cDNA clones (vs. 8 of 16 AT genomic clones) and 8 of 10 normal cDNA clones (vs. two of seven normal genomic clones) maintained correct open reading frames. This dramatic predominance of productive rearrangements in the expressed hybrid genes compared with the occurrence at the DNA level demonstrates enhanced expression of potentially functional receptors. Normal Ig and TCR genes also exhibit preferential expression of productively rearranged genes (30, 33–35), and this is presumably driven by selection of cells bearing functional

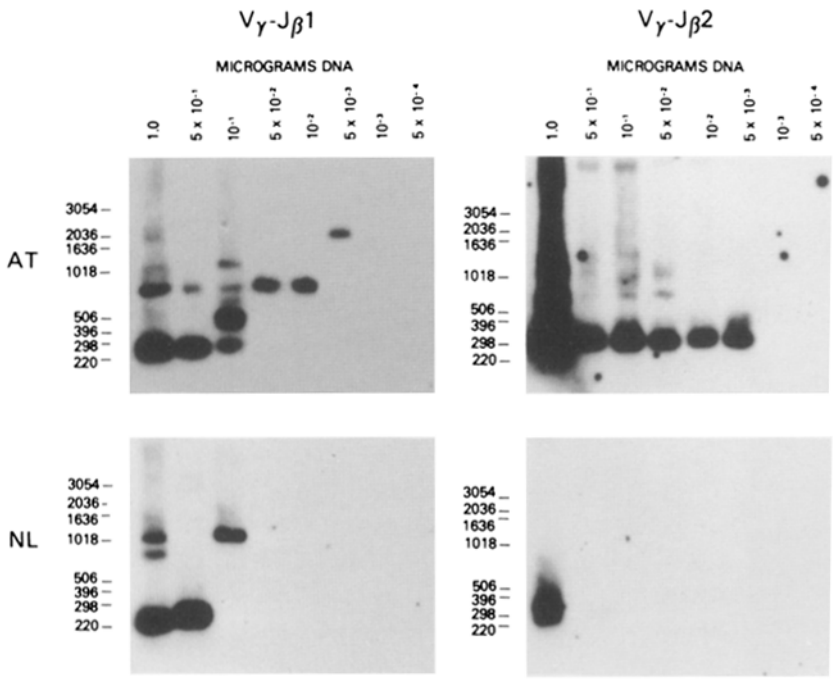

Figure 3. Southern blot analysis of amplified Vγ-Jβ hybrid genes from serially diluted genomic PBL DNA from an AT patient (AT) and a normal individual (NL). Left panels are amplification of Vγ-Jβ1 hybrids, and right panels are amplification of Vγ Jβ2 hybrids from an AT patient (*top*) and a normal individual (*bottom*). Size markers are in base pairs. Amount of DNA (in micrograms) added per PCR reaction is shown at the top. The blots were hybridized to the Vγc oligonucleotide probe. The apparent paradoxical increased yield of larger products at lower DNA concentrations resulted from the inefficient amplification of large products in the presence of competing small products (i.e., *top left*). Increased yield of the larger products resulted when the DNA was diluted to a point that the small product was no longer present. Longer exposure of the autoradiograph revealed faint bands corresponding to the larger product in the first five lanes (data not shown).

antigen receptors. We also observed a predominance of Vγ 1.4 segments utilized in the expressed hybrid genes. This bias toward cDNA clones that maintain an open reading frame and utilize a specific Vγ region, despite no such bias in the genomic clones, supports a functional role for the encoded hybrid antigen receptor proteins. The previously described naturally occurring hybrid gene resulting from a site-specific recombination between an Ig H chain V region and TCR-α J region in a lymphoma cell line has been shown to result in expression of the hybrid protein (I.R. Kirsch, C.R. Rittershouse, T-cell Sciences, Cambridge, MA; unpublished data). Artificially constructed hybrid genes that combined antigen-specific Ig V-J regions with TCR-α or TCR-β C regions have been shown to result in expression of the molecule on the cell surface, to result in association of the chimeric protein chain with the endogenous TCR β or α chains, and to result in a functional antigen-specific response in both transfected T cell lines (36, 37) and in transgenic mice (38). Taken together, these data indicate that the hybrid genes that result from interlocus recombination can in fact result in functional chimeric TCR heterodimers that may serve to increase the TCR repertoire.

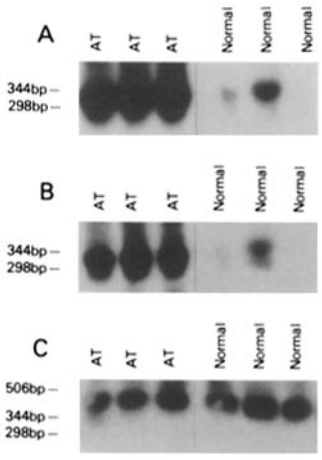

Figure 4. Southern blot analysis of hybrid genes amplified from cDNA of AT patients (AT) and normal individuals (NL). First-strand cDNA prepared from PBL RNA and amplified with PCR primers specific for hybrid genes was hybridized to Vγc oligonucleotide probe (*A*) or stripped and rehybridized to Cβc oligonucleotide probe (*B*). Longer exposure revealed that all normal samples were positive, while control cDNA prepared from the SUP-T1 cell line was negative (data not shown). First-strand cDNA amplified with PCR primers specific for rearranged TCR-β was hybridized to the Cβc probe (*C*).

An unusual class of cDNA clones showed intralocus recombination between Vγ and Jγ regions and splicing of the Vγ-Jγ exon to a Cβ exon (Table 3). This structure could be generated by interlocus recombination after the normal intralocus Vγ-Jγ rearrangement had already occurred, although the recombination signals utilized in the other interlocus rearrangements described in this paper would no longer be present to mediate the rearrangement, and the mechanism and driving force for such a secondary rearrangement is therefore unclear. Alternatively, this structure could result from *trans*-splicing of the mRNA between functional transcripts from the rearranged TCR-γ locus and nonfunctional sterile transcripts, which precede rearrangement (33) from the unrearranged TCR-β locus. Such a mechanism of *trans*-splicing of Ig genes has been previously postulated (39). One model that would explain the propensity of these two loci to both recombine and *trans*-splice might be that they are in close proximity to one another in the interphase nucleus. This would support

Table 3. *Sequences of V-J Junctions of cDNA Clones*

Clones	V	N	J	Open reading frame
AT				
1	(Vγ 1.4) **GCCACCTGGGATGGG**	AG	TCAG**CCC** (Jβ 1.5)	+
2	(Vγ 1.2) **GCCACCTGGGACGG**	CGTCGGGGACCTCGACGGTAA	C**TAC** (Jβ 1.2)	+
3	(Vγ 1.4) **GCCACCTGGGATG**	AA	GAGGACCTG**AAA** (Cβ 2)	NA‡
4*	(Vγ 1.3) **GCCACCTGGGACAGG**	CCCCTGTCGATG	GAT**ACG** (Jβ 2.3)	+
5	(Vγ 1.4) **GCCACCTGGGAT**	TCA	TCCTACGAG**CAG** (Jβ 2.7)	+
6	(Vγ 1.5) **GCCACCTGGGA**	AAGGGATCTCG	GCACAGAT**ACG** (Jβ 2.3)	+
7	(Vγ 1.2) **GCCACCTGG**	TCCCAGGGGTGGATAA	TACGAG**CAG** (Jβ 2.7)	−
8	(Vγ 1.4) **GCCACCTGGGATG**	T	T**GAG** (Jβ 2.1)	+
9	(Vγ 1.4) **GCCACCTGGGATGG**	ATTTTCT	AGCACAGAT**ACG** (Jβ 2.3)	+
10	(Vγ 1.4) **GCCACCTGGG**	TGAT	CTACGAG**CAG** (Jβ 2.7)	+
11	(Vγ 1.4) **GCCACCTGGGATAGG**	CG	TAAT**TCA** (Jβ 1.6)	+
12	(Vγ 1.4) **GCCACCTGGGACAGG**	GTGAGC	TCCTACGAG**CAG** (Jβ 2.7)	+
13	(Vγ 1.4) **GCCACCTGGG**	TACTA	CAAGAGACC**CAG** (Jβ 2.5)	+
Normal				
1	(Vγ 1.3) **GCCACCTGGGAC**	CGCCCCA	GAA**AAA** (Jβ 1.4)	−
2*	(Vγ 1.3) **GCCACCTGGGAC**	CTCGCCAGG	GGC**TAC** (Jβ 1.2)	+
3*	(Vγ 1.4) **GCCACC**	CCTAGG	AGTAGTGAT**TGG** (Jγ 2.1)	+§
4*	(Vγ 1.8) **GCCACCTGGGATGGG**	AAC	TCTGGAAAC**ACC** (Jβ 1.3)	+
5	(Vγ 1.4) **GCCACCTGGGATGGG**	CCTTTGGT	TGGGGCC**AAC** (Jβ 2.6)	+
6	(Vγ 1.4) **GCCACCTGGGATGGG**	CGGAG	TGGAAAC**ACC** (Jβ 1.3)	+
7	(Vγ 1.4) **GCCACC**	AGTTGG	GAGGACCTG**AAA** (Cβ 2)	NA‡
8	(Vγ 1.4) **GCCACCTGGGATGGG**	A	AAGAGACC**CAG** (Jβ 2.5)	+
9	(Vγ 1.4) **GCCACCTGGGATGGG**	CGTGG	TGAT**TGG** (Jγ 2.1)	+§
10	(Vγ 1.3) **GCCACCTGGGATGG**	-	CTGAA**GCT** (Jβ 1.1)	−
11	(Vγ 1.4) **GCCACCT**	TGG	AAGAGACC**CAG** (Jβ 2.5)	+

See legend for Table 2. All clones sequenced were correctly spliced to a Cβ segment (data not shown.)
* Multiple identical clones were found.
‡ No J region was identified and so open reading frame is not applicable (NA). These are discussed further in the text.
§ These clones recombined Vγ and Jγ segments, which were then spliced to Cβ1. These are discussed further in the text.

the concept of a structural or functional organization of the interphase nucleus (40, 41).

Another unusual class of cDNA clones had no Jβ region, but instead had a Vγ-N-Cβ structure (Table 3). This cDNA structure could be generated by the creation of an RNA splice donor at or within the N-region. The occurrence of two such cDNA clones from different RNA samples suggests that these clones represent mRNA species present in the cells. To our knowledge, no such cDNA structure has been previously reported.

A recent study by Tycko et al. (42) demonstrated recombination between TCR-γ and TCR-δ genes in normal thymocytes. No data were reported from patients with AT. However the structure of the V-J junctions, the frequency of their formation, and the predominance of in-frame rearrangements in the cDNA vs. genomic clones were analogous to our results on the formation of TCR-γ-β hybrids.

There is inferential data supporting the idea that the predisposition of AT patients to develop lymphoid malignancies may be related to the same process that leads to their exaggerated formation of hybrid TCR genes. In addition to the high frequency of hybrid antigen receptor gene formation in AT, there is a high frequency of the development of clonal proliferations of lymphocytes containing specific cytogenetic abnormalities involving chromosome 14 (43–45). Molecular analysis of these clonal abnormalities revealed a mechanism of rearrangement similar to hybrid antigen receptor formation. These rearrangements have as one partner a TCR J region that recombines in a site-specific fashion with a region of chromosome 14 (14q32.1) that has potential recombination signals within its sequence (46–48). This same type of chromosomal aberration has been shown to be highly associated with the development of T cell prolymphocytic leukemia in normal adults (49). This region of chromosome

14 may contain a putative growth-affecting gene that presumably could be misregulated by the translocation. The increased frequency and related structure of hybrid antigen receptor formation and T cell clonal abnormalities in AT patients suggests that the same mechanism underlies the occurrence of both. Studies of this predisposition to form hybrid antigen genes thus may also provide insight into the mechanism of translocations between antigen receptor genes and growth-affecting genes.

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