Inversion of Chromosome 7 in Ataxia Telangiectasia Is Generated by a Rearrangement Between T-Cell Receptor β and T-Cell Receptor γ Genes

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Specific and recurrent chromosomal rearrangements are often observed in the karyotypes of phytohemagglutinin-stimulated lymphocytes. The percentage of cells demonstrating these rearrangements is dramatically increased in the genetic disease ataxia telangiectasia. Inversion of chromosome 7 represents approximately half of the chromosomal rearrangements in this disease. Because the chromosomal locations of the inv(7) breakpoints coincide precisely with those of the T-cell antigen receptor (TCR) β and γ genes, it has been hypothesized that this rearrangement may occur by recombination between these two loci. Here, we present direct evidence that inversion of chromosome 7 in ataxia telangiectasia is generated by site-specific recombination between a TCRγ variable segment and a TCRβ joining segment. This is a US government work. There are no restrictions on its use.

MATERIALS AND METHODS

Patient and T-cell culture. The AT patient was described previously. Peripheral blood lymphocytes were isolated from heparinized blood by Ficoll-Hypaque (Pharmacia Fine Chemical, Uppsala, Sweden) density sedimentation. Cells were washed and resuspended in RPMI 1640 at a concentration of 10^6 cells/mL (GIBCO, Paisley, England) supplemented with glutamine (2 mmol/L), human serum AB⁺ (10%), crude T-cell growth factor⁵ (10%), and 5 × 10^8/mL irradiated (4,500 rad) allogeneic mononuclear cells. Cells were grown at 37°C in a 5% carbon dioxide incubator. After an initial PHA stimulation (15 μg/mL, Difco, Detroit, MI), the cells were restimulated every 10 days by allogeneic irradiated feeder cells and medium was changed twice a week. A different feeder layer was used each time to prevent development of allogeneic T-cell populations. R-banded karyotypes were performed according to our standard technique. DNA extraction and Southern blot analysis. High-molecular-weight DNA was extracted, digested to completion by various restriction enzymes according to the recommendation of the supplier (Genosol, Paris, France), electrophoresed in a 0.8% agarose gel, and transferred to a Nylon membrane (Zetabind, Cuno, Meriden, CT). The genomic blot was successively hybridized to different probes with an intensifying screen (Dupont Cronex), and stripped in 0.5 N NaOH before the next hybridization.

The TCRβ constant probe (CB) is the 770-base pair (bp) PstI fragment of the Jurkat TCRβ cDNA. The TCRγ variable probe (Vγ) is the 1.2-kilobase pair (kb) EcoRI-SacI fragment containing a genomic rearranged Vγ1.3-J γ1.2. The TCRγ joining probe (Jγ) is the 2.1-kb HindIII fragment from M13H60. DNA amplification by the Taq polymerase. Amplification with the Taq polymerase was performed according to the supplier (Perkin Elmer Cetus, Norwalk, CT). Target sequences were amplified in a 100-μL reaction buffer containing 1 μg genomic DNA, 50 mMol/L KCl, 10 mMol/L Tris HCl, pH 8.3, 1.5 mMol/L MgCl₂, 0.01% (wt/vol) gelatin, 200 μmol/L of the four deoxynucleotide triphosphates, 1 μmol/L of each primer, and 2.5 U Taq polymerase. Sources of genomic DNA included the inv(7) cell line, the peripheral blood lymphocytes and purified T lymphocytes from the AT patient and, as controls, placental DNA (Clontech, Palo Alto, CA) and DNA from the SUP-T1 malignant T-cell line. The samples were incubated at 94°C for 1 minute to denature the DNA, cooled at 60°C for 2 minutes to allow primer annealing, and heated at 72°C for 3 minutes to allow extension by the Taq polymerase. The high annealing temperature was necessary to avoid amplification of nonspecific sequences. Thirty to 40 thermal cycles were performed with a Perkin Elmer Cetus DNA Thermal Cycler. Products of amplifications were analyzed on a 3% agarose gel after ethidium bromide staining.
bromide staining and subsequently transferred to a nylon membrane as described above.

Oligonucleotides used for the amplification. Oligonucleotides were synthetized on an Applied Biosystems 380B (Foster City, CA) DNA synthetizer. They were purified on an 8% denaturing acrylamide gel and, after electroelution, their concentrations were estimated by ethidium bromide staining on a 2% agarose gel. The following oligonucleotides were used for the polymerase chain reaction (PCR): Vγ, TACATCCACTGTACCTACACCAG 3' 24mer; Jβ2.7, CTCACCTGTACCGTGAGCC 3' 20mer; Jβ2.1, CCTACC TAGCACGGTGAGCC 3' 20mer.

Subcloning and sequencing the PCR fragments. The PCR products were electrophoresed on a 5% acrylamide gel and purified by electroelution. The ends of the fragments were polished with the T4 DNA polymerase and subcloned into the SmaI site of M13mp18 or mpl9. The fragments were sequenced in both directions using the dideoxy-chain termination method with the M13 universal primer.

RESULTS

Isolation of an inv(7) cell line. T lymphocytes from an AT patient were grown in a conditioned medium for more than 1 year. After an initial stimulation with phytohemagglutinin, restimulations were performed by washing the cells and plating them in fresh conditioned media with allogeneic irradiated mononuclear cells. During the first 3 months of culture, no growth was observed and the repeated karyotypes were unremarkable. A fast-growing population was then observed; karyotypes at that time showed more than 90% of the metaphase cells bearing an inv(7) (p14q35) rearrangement (Fig 1). However, this inv(7) cell line was highly unstable in culture. Attempts at cloning or further expanding the cell line were unsuccessful. Only 1% to 10% of the cells carried the inversion after an additional month of culture.

Southern blot analyses. Rearrangements of the TCRβ and TCRγ loci were searched for by Southern blot analysis. Using a Cβ probe on HindIII digest, a 7.5-kb abnormal fragment was observed in addition to the three germline fragments (Fig 2). Using a Vγ probe, a nongermline band of the same size was observed in addition to the germline bands (Fig 2). A comigrating 20-kb fragment was also detected by both probes in a BamHI digest (data not shown). Furthermore, the rearrangement detected with the Vγ probe on both digests does not hybridize to the Jγ probe as might have been expected if it were a VγJγ rearrangement. However, in addition to the 2.1-kb fragment (germline Jγ1) and the 5-kb fragment (germline Jγ2), two rearranged bands were detected. Because they comigrated with Vγ bands, these fragments were interpreted as Vγ-Jγ rearrangements. The residual germline bands detected in the inv(7) DNA with all the probes were believed to be due to polyclonal cells present in the inv(7) cell line.

DNA amplification of the rearrangement. To investigate the possibility of a VγJβ rearrangement as had been suggested by the Southern blot analyses, we synthesized a consensus oligonucleotide for the TCRγ variable gene family I (Vγ) and oligonucleotides that were complementary to...
TCRβ Jβ2.1 and Jβ2.7 joining regions. Amplification using the Taq polymerase and different combinations of Vγ and Jβ oligonucleotides was then performed on genomic DNA from the inv(7) cell line. Controls for the specificity of the reaction included DNA from a T-cell line and from human placenta. A 250-bp fragment was observed only when Vγ and Jβ2.1 oligonucleotides were used as primers on genomic DNA from the inv(7) cell line. After transfer to a nylon membrane, these fragments were identified by both Jβ and Vγ probes. No such positively hybridizing product was observed in the controls (data not shown).

Sequencing the PCR products. The PCR fragment amplified from the inv(7) cell line was sequenced in both directions (Fig 3). This sequence was compared with the published sequences for the TCRβ and TCRγ loci.18,19 The fragment was precisely homologous to the sequence of TCRγ Vγ1.3 up to four bases 5’ of the heptamer sequence of the germline gene. After five bases that could not be assigned, the fragment became homologous to the Jβ2.2 region.

Cloning and sequencing the inv(7) breakpoint from the PBLs. To demonstrate that this particular inv(7) rearrangement was present in the T lymphocytes of the AT patient and did not occur in vitro, we amplified the inv(7) breakpoint from two different samples of genomic DNA from the AT patient from whom the cell line had been derived. The DNAs were from fresh PBLs and from a CD4+CD8+ T-cell population in which we had previously demonstrated a high frequency of inv(7).8 Using the same PCR protocol and the same primers, we observed a 250-bp fragment. The sequence of this fragment was identical to that identified in the inv(7) cell line (Fig 3). The placenta and the T-cell line DNA controls examined simultaneously were negative for this amplified product.

DISCUSSION

An important biologic distinction between AT patients and normal individuals is the increased frequency in AT of recurrent chromosomal translocations and inversions occurring in a cell type specific pattern. In T cells, these chromosomal aberrations occur within the chromosomal regions which also encode the primary differentiated products of these cells, the TCR genes.

We showed that one such aberration, an inversion of chromosome 7 occurring in the T cells of an AT patient, is generated by a recombination between two genes of the TCR family. The sequence of the rearrangement corresponds to an apparent site-specific recombination between Vγ1.3 and Jβ2.2. The five nucleotides at the junction have no homology to Vγ1.3 or Jβ2.2 and are likely to be an N-diversity region. Neither Southern blot analyses nor attempts to amplify the predicted reciprocal product defined the presumed reciprocal rearrangement. This is suggestive of a secondary rearrangement, as was reported in another analogous system.20

Inversion of a segment of DNA to generate a V–J junction is a physiological mechanism of recombination that has been described in the mouse TCRβ21 and in the human TCRβ.22 This inv(7) differs from those examples only by the huge distance that separates the Vγ and the Jβ regions. Because of the position of the TCRβ and TCRγ genes on chromosome 7, and with the knowledge that the TCRβ constant regions are telomeric to their TCRβ joining regions,23 we can deduce the orientation of the TCRG. The TCRγ constant regions must be telomeric to the TCRγ variable regions as illustrated in Fig 4.

Chromosomal rearrangements such as this inv(7) occur in only a small fraction of the peripheral blood lymphocytes. The characterization presented here has been possible only by the use of the PCR technique. The extraordinary amplification capability of this technique allowed us to clone and sequence this rearrangement in a cell line derived from an AT patient. Furthermore, we were able to demonstrate the same rearrangement in the peripheral blood of this AT patient. Whether this particular inv(7) in this cell line represents an expansion in vitro of an inv(7) population that is also predominant in vivo is still unclear. This demonstration ruled out that the inv(7) occurred during cell culture. In
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REFERENCES

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