A chromosome 14 inversion in a T-cell lymphoma is caused by site-specific recombination between immunoglobulin and T-cell receptor loci

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Specific chromosomal aberrations are associated with specific types of cancer (for review see ref. 1). The distinctiveness of each association has led to the belief that these chromosomal aberrations are clues to oncogenic events or to the state of differentiation in the malignant cell type2.3. Malignancies of T lymphocytes demonstrate such an association characterized most frequently by structural translocations or inversions of chromosomes 7 and 14 (refs 7-9). Analyses of these chromosomally marked tumours at the molecular level may therefore provide insight into the aetiology of the cancers as well as the mechanisms by which chromosomes break and rejoin. Here we report such an analysis of the tumour cell line SUP-T1 derived from a patient with childhood T-cell lymphoma carrying an inversion of one chromosome 14 between bands q11.2 and q32.3, that is10, 11q11.2; q32.3). These are the same chromosomal bands to which the T-cell receptor-α chain (11q11.2) and the immunoglobulin heavy-chain locus (14q32.3) have been assigned. Our analysis reveals that this morphological inversion of chromosome 14 was mediated by a site-specific recombination event between an immunoglobulin heavy-chain variable region (IgV H) and a T-cell receptor (TCR) α-chain joining segment (TCCRα). Sí recombination analysis shows that this hybrid gene is transcribed into polycistronic (poly[A] RNA).

We began our analysis by studying the organization and structure of the TCR-α chain locus in the SUP-T1 cell line. The germline genomic structure of the TCR-α chain locus comprises a single constant region (Cα) preceded 5' by several joining segments (Jα) dispersed over at least 30 kilobases (kb)11. The variable regions (Vα) reside at an unknown distance more centromeric to the TCR Cα locus within the same chromosomal band12,13. Using DNA probes containing germline TCR Jα regions spanning 15 kb 5' of TCR Cα, two distinct TCR Cα-linked rearrangements were detected in this cell line. Probe A, from the region 5 kb 5' of TCR Cα, demonstrated a rearrangement seen as a novel 8-9 kb band on HindIII digestion of SUP-T1 genomic DNA (Fig. 1a). Germline 4.3- and 3.6-kb bands were identified with this probe. A second TCR Cα-containing probe, probe B, derived from a region 15 kb farther upstream of TCR Cα, identified a second rearrangement, a novel 2.5-kb band on EcoRI-digested genomic DNA (Fig. 1b). With probe B, no germline band was seen in SUP-T1, suggesting that this sequence had been deleted from the allele which contained the more 3' rearrangement. A germline probe taken from a region 2.5 kb farther 5' did not detect any homologous sequences in SUP-T1.
DNA, indicating that this region was deleted in both alleles (data not shown).

The S-most C3′-linked \( J_e \) rearrangement was cloned by screening a partial MboII SUP-T1 genomic DNA library with probe B. A comparison with germline TCR \( J_e \) map allowed precise localization of the region in which recombination had occurred. In this case, however, sequence analysis (Fig. 2b) did not reveal the conventional \( V_e/J_e \) joining, but instead a site-specific recombination event that joined a completely intact immunoglobulin heavy-chain variable region (Ig \( V_e \)) to the TCR \( J_e \) segment. Translation of this sequence shows that the Ig \( V_e/\)TCR \( J_e \) join has an open reading frame for the complete coding region. There is a complete amino-acid sequence homology between the framework regions 1, 2 and 3 of this variable-region gene and sequenced proteins of Ig \( V_e \) subgroup II (ref. 18). In contrast, there is no extensive homology between this gene and TCR \( V_e \) genes sequenced by ourselves or others.

Numerous groups have definitively localized the TCR \( \alpha \)-chain gene to 14q11.2 (refs 11, 12) and the immunoglobulin heavy-chain variable and constant regions to 14q32.3 (refs 13, 20 and 21).

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I.R.K., C. C. Morton and J. V. Ravetch, unpublished data). The orientation of these loci is also known: variable regions are telomeric of constant regions in the immunoglobulin genes and variable regions centromeric of constant regions in the T-cell receptor \( \alpha \)-chain locus. Thus, Ig \( V_e/\)TCR \( J_e \) recombination would result in chromosomal inversion (Fig. 3), precisely the morphological chromosomal aberration seen in this cell line. Chromosomal mapping studies in analogous systems support this model.

We examined possible transcriptional activity of both TCR \( V_e/\)J\(_e\) and Ig \( V_e/\)TCR \( J_e \) rearrangements by \( S_{\alpha} \) nucleic analyses. Single-stranded \( ^32\)P-labelled M13 probes complementary to the TCR \( V_e \) and Ig \( V_e \) genes were prepared and hybridized to total and poly(A)\(^+\)-enriched RNA from SUP-T1 and control cells and subsequently digested with \( S_{\alpha} \) nuclease\(^2\). Because the TCR \( V_e \) probe contains only TCR \( V_e \) coding sequence (Fig. 4a), active transcription of this gene should afford full-length protection of this probe. \( S_{\alpha} \) analysis of SUP-T1 RNA revealed a 200-bp protected fragment consistent with transcription of this TCR \( V_e \) gene.
Fig. 3. A model for inversion of chromosome 14 via Ig V_{\gamma}2-TCR J_{\gamma} site-specific recombination. The chromosomal band localization of the immunoglobulin and T-cell receptor loci and their orientation are shown, as is the upper idiogram of a normal chromosome 14. Site-specific recombination between an immunoglobulin heavy-chain variable region (V_{\gamma}2) and a T-cell receptor \(\alpha\)-chain joining segment (J_{\alpha}) results in an inversion of chromosome 14 at bands q11.2 and q32.3 (vertical dotted lines), as shown in the lower idiogram. The additional TCR V_{\alpha}2-J_{\alpha} recombination in the inverted chromosome in the schematic diagram was identified by cloning experiments (data not shown). The orientation of the immunoglobulin and T-cell receptor loci in this scheme is based on the cloning experiments presented here (see test). Hatched region, sequences from band q32.3; open region, sequences from band q11.2.

Fig. 4. S_{\gamma} nucleoside analysis of SUP-T1 RNA. a, Transcription of a TCR V_{\gamma} gene. An S_{\gamma} probe (solid bar) was prepared from 200 bp of internal TCR V_{\gamma} coding sequence and 40 bp of M13 primer. Left-hand lane, control (no RNA); right-hand lane, SUP-T1 poly(A) + RNA. When annealed to poly(A) + RNA, all 200 bases of TCR V_{\gamma} coding region (stippled region in the schematic diagram) were protected from S_{\gamma} nucleoside digestion. b, Transcription of an immunoglobulin V_{\gamma} gene. An S_{\gamma} probe (solid bar) was prepared from 270 bp of Ig V_{\gamma} coding and 5' intervening sequence. Left lane, control (no RNA); right lane, SUP-T1 poly(A) + RNA. When annealed to poly(A) + RNA, the 120 bases of the Ig V_{\gamma} coding sequence were protected from S_{\gamma} nucleoside digestion. The hatched region in the schematic diagram is the entire Ig V_{\gamma} coding sequence.

The Ig V_{\gamma}-TCR J_{\gamma} recombinant described here clearly demonstrates that one recombination system22-26 can recognize both immunoglobulin20 and T-cell28-31 substrates and unify them; it also requires the cell to have been in a developmental state where these two distinct and disparate loci were both susceptible to a recombination event. The spacing of the signal sequences 5' of the TCR J_{\gamma} and 3' of the Ig V_{\gamma} suggests that the joining event would not require interposition of a 2 J_{\gamma} (diversity) segment if previously postulated rules of joining31 pertained. Current hypotheses suggest that these recombinations may also mediate recombination between immunoglobulin loci and other more divergent genes and signal sequences, possibly resulting in the t(14; 18)22,23 or (11; 14)34 translocations seen in subgroups of B-cell tumours.

The chromosome 14 inversion has been observed only in frankly malignant clones of T cells30,31, including four of five recently described patients with T-cell chronic lymphocytic leukaemia, or in 'normal' T cells from patients with diseases that predispose to the development of leukemia or lymphoma, such as ataxia telangiectasia35-37. Although this karyotypic finding may only reflect the aberrant joining of two distinct loci by a common recombination system at a particular point in lymphocyte development, it is also possible that this chromosomal alteration presages or contributes to malignant transformation of T cells. This might occur by activation of an as yet unidentified oncogene, or perhaps by the formation of a hybrid immunoglobulin-TCR cell-surface receptor providing an inappropriate mitogenic stimulus.

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