The EMBO Journal vol.6 no.8 pp.2273-2280, 1987

Human T-cell tumours containing chromosome 14 inversion or translocation with breakpoints proximal to immunoglobulin joining regions at 14q32

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Communicated by T.H.Rabbitts

T-cell tumours are frequently found to carry an inversion of chromosome 14 (inv(14)) (q11;q32) or more rarely a chromosome 14 translocation t(14;14) with the same cytogenetic breakpoints (q11;q32). We have examined the molecular junctions of an inv(14) and a translocation t(14;14) using T-cell receptor (TCR) α joining (J) region probes. Both of these chromosomal abnormalities have breakpoints within the TCR J_α locus at 14q11 and both have breakpoints which are proximal (i.e. on the centromeric side) to the immunoglobulin heavy chain J_H region at 14q32. The cloned segments corresponding to the junctions at 14q32 are not associated with obvious immunoglobulin-like sequences. This contrasts to the previously described inv(14) in the cell line SUP-T1 and places a potential cluster of chromosome 14 breakpoints downstream of the Ig J_H locus. The possible role of the varying breakpoints in the development of these tumours is discussed.

Key words: chromosome inversion/immunoglobulin/T cell/TCRα/translocation

Introduction

Tumour-specific chromosomal abnormalities such as translocations, inversions or deletions, are a common feature of human lymphoid neoplasia (reviewed by Yunis, 1983). This is well exemplified in the chromosomal translocations found in Burkitt's lymphoma (reviewed by Klein and Klein, 1985). The consistent presence of such abnormalities is considered to be an indication that the development of this tumour is brought about by oncogene activation resulting directly from the chromosome abnormality. T-cell leukaemias frequently carry one of two types of chromosome 14 change; either inversion of the long arm with breakpoints at q11 and q32 or translocation between the allelic chromosomes 14 but with cytogenetically similar breakpoints to those of the inv(14) (i.e. q11; q32) (McCaw *et al.*, 1975; Taylor *et al.*, 1981; Hecht *et al.*, 1984; Uieshima *et al.*, 1984; Zech *et al.*, 1984; Sadamoari *et al.*, 1985; Clare *et al.*, 1986). Tumours with inv(14) retain a normal chromosome 14 whereas those with t(14;14) only have the two reciprocal translocated chromosomes. Similar chromosomal interchanges have also been described in apparently non-leukaemic, but nonetheless clonal, proliferations in patients suffering from ataxia telangiactasia (Taylor *et al.*, 1981; Aurias *et al.*, 1986). In one case, a clone carrying inv(14) has been followed through from this non-malignant status to an overt T-CLL (Taylor and Butterworth, 1986).

A molecular analysis of the chromosomal breakpoints in a number of tumours with q11;q32 abnormalities is called for in order to establish the existence of any consistent features which might shed light on the role of the q11 and q32 junctional chromosomal segments in T-cell leukaemia. Two examples have been studied so far. One is a cell line (SUP-T1) derived from a T-cell lymphoma patient carrying inv(14) (Baer *et al.*, 1985; Denny *et al.*, 1986a) and the other is a non-malignant clone from an ataxia telangiactasia (A-T) patient with translocation t(14;14) (Kennaugh *et al.*, 1986). In the former case, molecular cloning studies have shown that the inv(14) chromosome was formed by breaks within the T-cell receptor (TCR) α locus at band 14q11 and within the variable (V) region segments lying on the distal (i.e. telomeric) side of the immunoglobulin (Ig) heavy (H) chain locus at band 14q32. This latter feature contrasts with the breakpoint observed at 14q32 in the t(14;14) of the A-T cell clone where the interchromosome junction occurs proximal (i.e. centromeric) to the Ig constant (C) region μ chain gene.

We have now examined the chromosomal breakpoints of a further case of an inv(14) chromosome and one case of a translocation t(14;14), both of which were isolated from T-cell tumours. We find that both tumour-associated abnormalities break within TCR J_α at 14q11 and, like the t(14;14) of the A-T clone, the 14q32 breakpoints occur proximal to the Ig H chain J_H region.

Results

Both alleles of TCRα are rearranged in the inv(14) and translocation t(14;14) tumour DNAs

DNA was prepared from tumour biopsy samples of a T-CLL carrying an inv(14) chromosome (Zech *et al.*, 1984) and a T-PLL carrying a translocation t(14;14) (designated Linv and Pt respectively). Both tumours have cytogenetic breakpoints mapping to 14q11 and 14q32. The previous observation that an inv(14) chromosome breaks within the J_α locus at chromosome 14 band q11 (Baer *et al.*, 1985; Denny *et al.*, 1986a), prompted a study of rearrangement of the J_α region in the two tumour DNAs. The J_α locus in mouse and man is very large (Hayday *et al.*, 1985; Winoto *et al.*, 1985; Yoshikai *et al.*, 1985) and so it is not possible to detect all rearrangements in T cells with, for example, a single TCRα constant (C) region probe or even several J_α region probes. We previously described two human J_α probes, separated by ~10 kb, from just upstream of the C_α gene (Baer *et al.*, 1985). These two probes plus two newly isolated ones (illustrated in Figure 1A) were used as probes in Southern filter hybridizations with the tumour DNA. Rearrangements on both allelic chromosomes were detectable with both samples. DNA from the translocation t(14;14) sample Pt showed both rearranged and unrearranged fragments in *Bam*HI- and in *Sac*I-cleaved DNA, when hybridized with the most downstream J_α locus probe, J_αBS (Figure 1B). On the other hand, only unrearranged fragments were seen in *Hin*dIII-digested DNA which

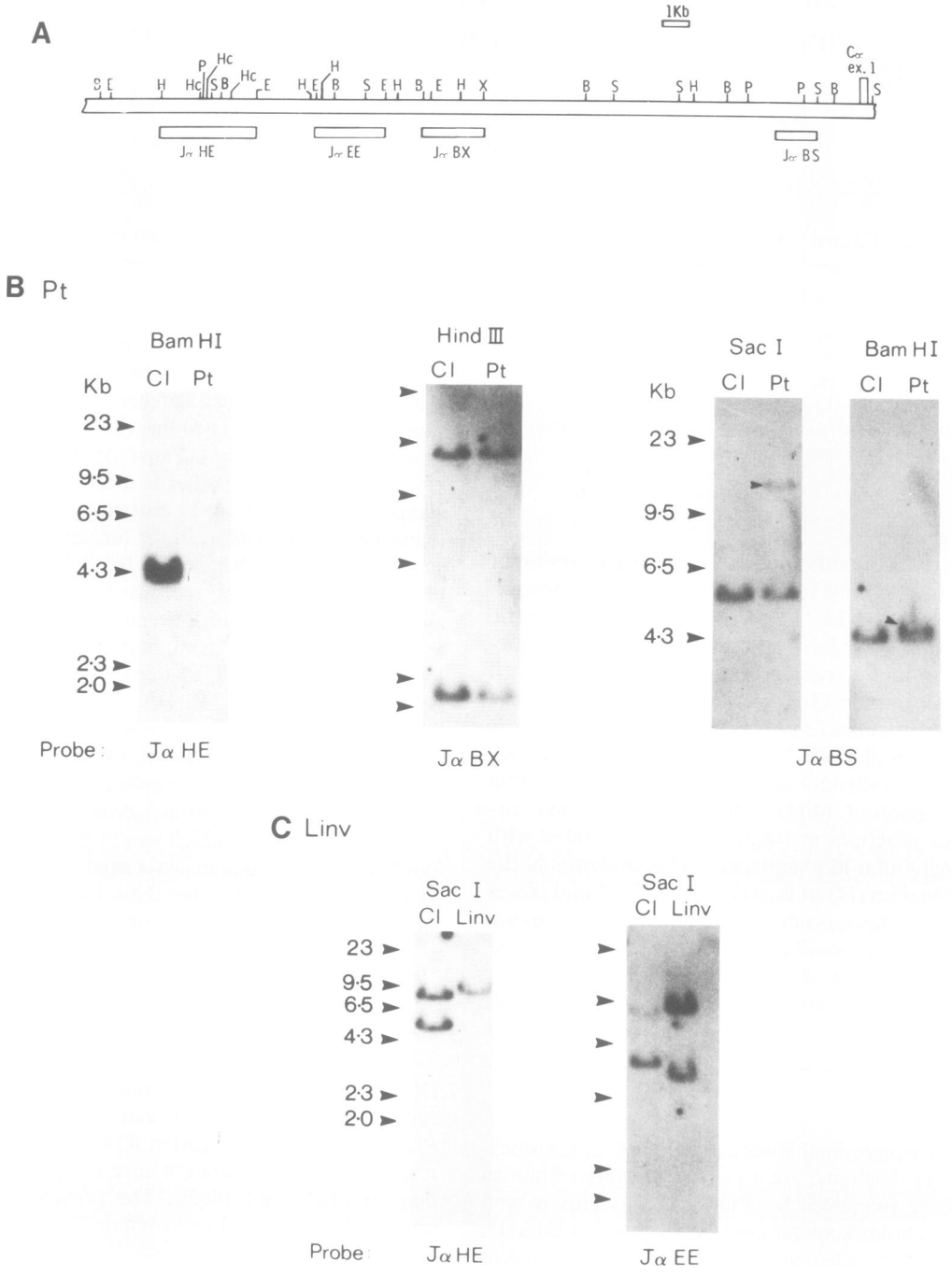

Fig. 1. Rearrangement of the TCR J_α locus in T-cell tumours. **(A)** Partial restriction map of TCR J_α locus at chromosome band 14q11. The positions of various probe fragments are shown as boxes beneath the restriction map which was produced by analysis of a number of overlapping, unrearranged λ phage clones (manuscript in preparation). Only the first exon of the C_α gene is shown. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; P, *Pst*I; S, *Sac*I; X, *Xho*I. Note: *Pst*I and *Hin*cII sites are incomplete. **(B)** Hybridization of J_α probes to Pt DNA. Southern filter hybridization of Cl [control DNA patient 7 (Rabbitts *et al.*, 1985)] and Pt DNA (cleaved with various enzymes) with various J_α probes as indicated. **(C)** Hybridization of J_α probes to Linv DNA. Hybridization of Cl and Linv DNA to J_α probes, as indicated. Sizes were determined by co-electrophoresis of λ DNA cut with *Hin*dIII.

was hybridized to J_αBX, while no hybridizable sequences were detectable in Pt DNA with J_αHE. This lack of hybridization indicates deletion of this DNA segment from both alleles which is a characteristic feature of rearrangements associated with V−J joining processes. The data therefore indicate that rearrangement on one Pt allele must have occurred within the 3-kb *Bam*HI−*Sac*I fragment detected by J_αBS and, on the other allele, in the 5.5-kb region between J_αBX and J_αHE.

Similarly, two J_α rearrangements were localized in Linv DNA, in this case usng J_αEE and J_αHE probes (Figure 1C). The J_αEE probe detects two fragments in *Sac*I-cleaved germline DNA; a faintly hybridizing 8.3-kb downstream band and a stronger hybridizing 5.2-kb upstream band (Figure 1A and C). The 5.2-kb fragment is absent from Linv DNA and instead we observe rearranged fragments of 4.8 and 8.4 kb (the faint germline 8.3-kb fragment is obscured by the latter rearranged

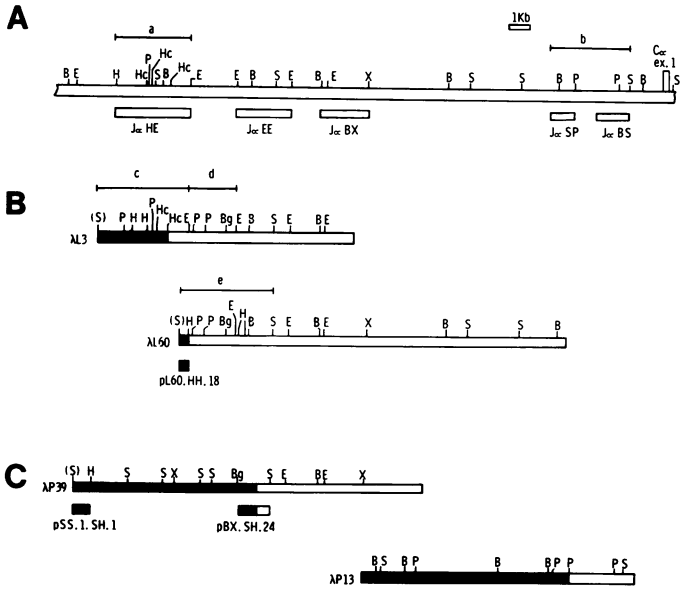

Fig. 2. Restriction maps of unrearranged and rearranged TCR J_α. **(A)** Partial map of unrearranged TCR J_α locus as in Figure 1A. **(B)** Restriction maps of rearranged clones derived from Linv DNA. *Bgl*II, *Pst*I and *Hinc*II sites are mapped only within overlined regions c–e. **(C)** Restriction maps of rearranged clones derived from Pt DNA. *Bgl*II sites are incomplete. In maps shown the open boxes represent TCR J_α locus DNA and the closed boxes represent rearranged DNA. Regions a–e which are overlined were mapped more finely than the basic λ maps. Relevant subclones in pUC vectors are indicated beneath the maps. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Hc, *Hinc*II; P, *Pst*I; S, *Sac*I; X, *Xho*I; Bg, *Bgl*II.

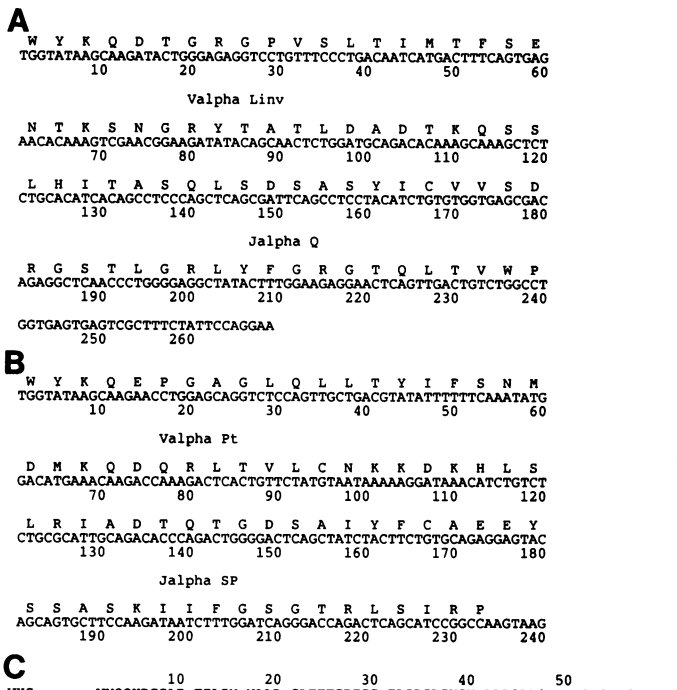

Fig. 3. Nucleotide sequences and derived protein sequences of productive alleles. Sequences are shown for Linv **(A)** and Pt **(B)** from the conserved W–Y dipeptide in the V_α regions. The cysteines putatively involved in disulphide bonds are circled. RNA splice sites are indicated by arrows. $J_\alpha Q$ has been previously described (Yoshikai *et al.*, 1986). **(C)** Comparison of protein sequence of $V_\alpha XS$ (Baer *et al.*, 1985) with those derived from $V_\alpha Pt$ and $V_\alpha Linv$. Dashes indicate identity of amino acids (which are shown in the single letter code).

fragment). Thus both J_α alleles in Linv are rearranged within the 5.2-kb germline fragment detected by $J_\alpha EE$. This was confirmed by hybridizing *Sac*I-digested DNA with $J_\alpha HE$ which detects the same 8.4-kb rearranged band in Linv DNA but no other germline or rearranged bands. Thus we conclude that one Linv J_α allele is rearranged between the 3′ end of $J_\alpha HE$ and the *Sac*I-site within $J_\alpha EE$ while the other is rearranged within the sequences covered by the $J_\alpha HE$ probe.

Productive TCR V_α–J_α and non-productive J_α rearrangements are present in both tumours

The organization of the various J_α region rearrangements was determined by studying isolated λ phage clones prepared from genomic libraries of the inv(14) and t(14;14) DNAs. Figure 2 shows comparative maps of the germline J_α locus, for ~26 kb upstream of the C_α gene (Figure 2A), and representative clones containing the J_α allelic rearrangements in the inv(14) sample Linv (Figure 2B) or the t(14;14) sample Pt (Figure 2C).

In the Linv DNA, the two J_α locus rearrangements occur within a 1-kb region on the allelic chromosomes. Restriction enzyme site mapping of the unrearranged J_α locus in $J_\alpha HE$ (region a, Figure 2A) compared with a subcloned *Sac*I–*Eco*RI fragment from λL3 (region c, Figure 2B) localized the rearrangement to within a 600-bp *Hinc*II fragment. The nucleotide sequence of this region revealed that a V-region gene had joined to a J_α segment (Figure 3A) and that the joining had occurred productively (i.e. such that the reading frame of the V and J_α were correctly aligned). The V segment involved in this rearrangement could be identified as belonging to the TCRα locus (i.e. 14q11) by alignment with another V_α sequence, $V_\alpha XS$ (Baer *et al.*, 1985). Although homologies are not good within the V_α families, sufficient similarity exists that assignment to this family is unequivocal (Figure 3C). No homology to Ig V_H segments could be found. Thus, this rearrangement appears to be a normal TCRα gene rearrangement resulting in a productive α chain gene.

The second J_α locus rearrangement in Linv DNA was located by mapping region e of λL60 (Figure 2B) and this showed, in comparison with germline region d, that a rearrangement had occurred between the *Hin*dIII and *Pst*I site (~180 bp apart) near the end of the λL60 clone. The nucleotide sequence of this small fragment was obtained and compared with the sequence from the equivalent unrearranged region of λL3 (GL, Figure 4A). This latter sequence reveals the presence of a J_α segment (designated J_α Lv in Figure 4A) with conserved heptamer–nanomer joining sequences; a *Pst*I site occurs near the 5′ end of this J_α segment. The J_α sequence defined in this region is compared in Figure 4C with two previously described J_α segments, $J_\alpha SP$ and $J_\alpha BX$, and shows several conserved residues within these segments. The restriction mapping of region e of λL60 (Figure 2B) showed tht the same *Pst*I site occurs just downstream of the point of rearrangement. Comparison of the relevant sequences (Figure 4A) shows identity between germline and rearranged genes to position 176 just upstream of the *Pst*I site; no sequence continuity is seen further upstream of this position. Therefore, new DNA has joined into the region immediately adjacent to the J_α Lv segment. However, examination of this sequence does not reveal any V-like sequence. Apparently, therefore, this rearrangement is a non-productive rearrangement of some type.

A similar type of analysis was carried out on the rearrangements detected in the translocation t(14;14) sample Pt.

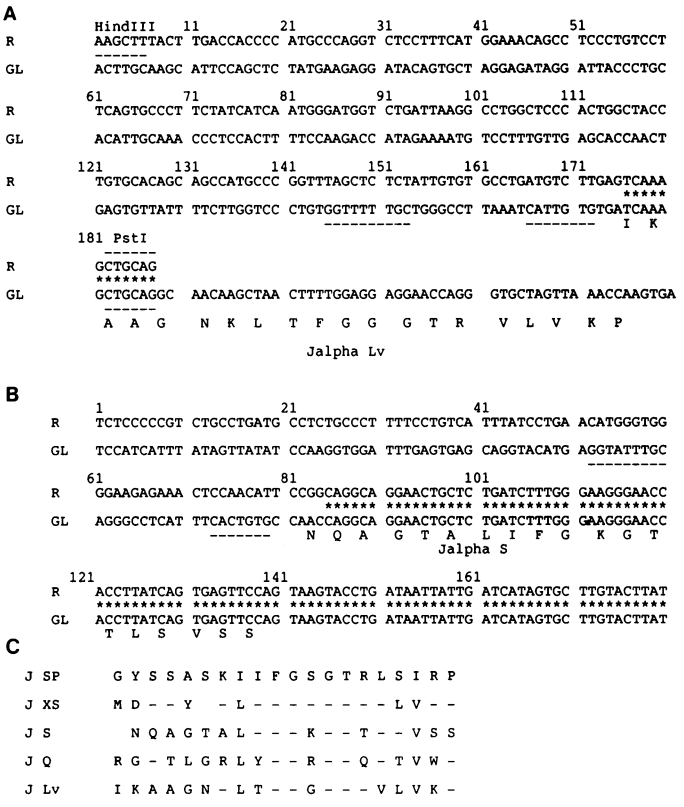

Fig. 4. Nucleotide sequences and derived protein sequences of non-productive alleles in the T-cell tumours. **(A)** Comparison of rearranged (R) and unrearranged (GL) segment of Linv DNA. The germline J_α Lv is shown with its derived amino acid translation. The conserved nanomer and heptamer joining signals are underlined. Relevant *Hin*dIII and *Pst*I sites are indicated which were utilized to sequence the rearranged breakpoint. Stars indicate sequence identity. **(B)** Comparison of rearranged (R) and unrearranged (GL) segments of Pt DNA. Annotations are as in **(A). (C)** Comparative J_α sequence [derived from Baer *et al.* (1985) J_αSP and J_αXS and this paper and Yoshikai *et al.* (1986)].

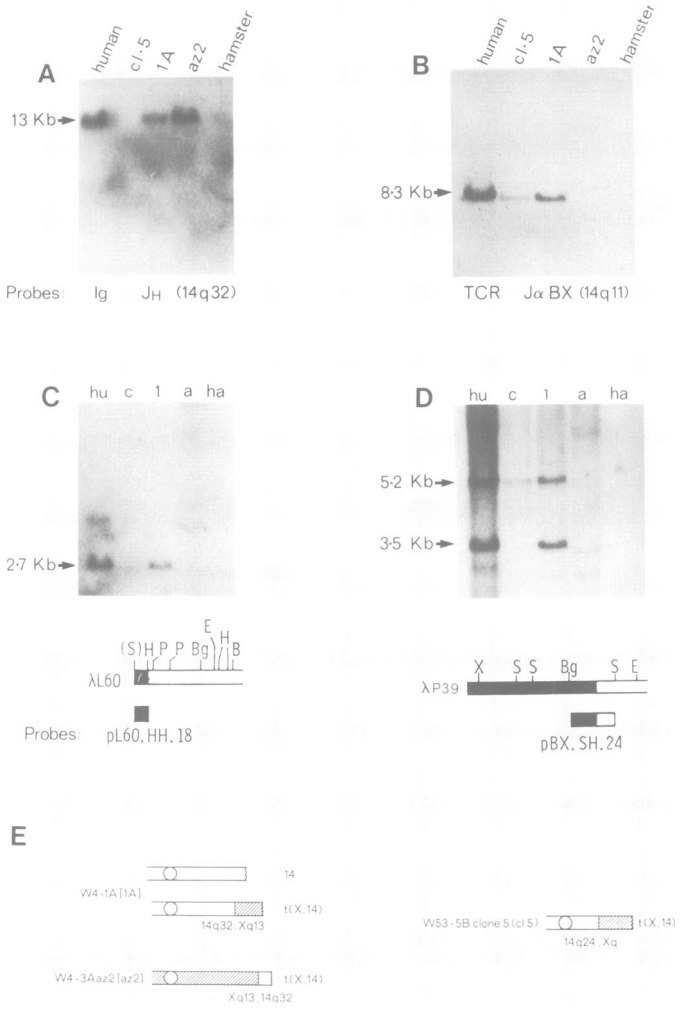

Fig. 5. Southern filter hybridization of somatic cell hybrid DNA. The cell hybrids used are described in the text. Human and hamster represent human and hamster DNA controls respectively. DNA (10 μg) of each cell type was analysed by Southern hybridization using various probes. The probes were **(A)** Ig J_H [M13C76R51A (Flanagan and Rabbitts, 1982b)], **(B)** TCR J_αBX (see Figure 1), **(C)** pL60.HH.18, **(D)** pBX.SH.24. S, *Sac*I; H, *Hin*dIII; P, *Pst*I; Bg, *Bgl*II; E, *Eco*RI; B, *Bam*HI. Sizes were calculated from co-electrophoresis of λ *Hin*dIII DNA. **(E)** Diagrammatic representation of cell hybrids used here.

The phage clones shown in Figure 2C are representative of the rearrangements found in Pt DNA. The rearrangement in λP13 occurs within a small *Pst*I fragment since such a fragment was not observed by mapping the unrearranged region b. Nucleotide sequencing of the 700-bp *Pst*I fragment from λP13, compared with the germline sequence in this region (Yoshikai *et al.*, 1985; Baer *et al.*, 1986), shows that a V gene has productively joined to J_αSP (Figure 3B). This V gene (VPt) was again found to belong to the V_α family since comparison with the V_αXS-derived protein sequence shows homology (Figure 3C), but no homology to V_H sequences could be found (unpublished data). Figure 3C (bottom) also shows a comparison between productively rearranged V_α isolated from Linv and Pt DNA. Within the regions compared, the two segments are ~38% homologous compared with ~30% homology to V_αXS (Figure 3C).

The rearrangement on the other allelic chromosome 14 in Pt DNA was mapped in λP39 to within a 1.4-kb *Bgl*II–*Sac*I fragment (Figure 2C). The *Sac*I site of the fragment is equivalent to that present in the germline J_α probe J_αEE. The precise position of rearrangement represented in λP39 was determined by comparing the nucleotide sequence of the *Sac*I–*Bam*HI fragment of J_αEE with the *Bgl*II–*Sac*I fragment corresponding to the insert of the pBX.SH.24 subclone of λP39. The relevant region of comparison is given in Figure 4B. The unrearranged germline sequence (GL) contains a J_α sequence (J_αS) as judged by the derived protein sequence compared with two previously described J_α segments (see Figure 4C) and the J_αQ and J_αLv described in this paper; the adjacent conserved heptamer–nanomer V–J joining signals are also shown in Figure 4B next to the J_α S segment. The sequence derived from the rearranged clone λP39 is also shown in Figure 4B (R). This sequence is identical to the J_α S sequence up to near the 5′ end of the J_α; thereafter the two sequences diverge. This, therefore, defines the rearrangement position in λP39. Examination of the protein translation of this sequence shows no homology to TCR or Ig-like sequence. Thus, like the rearrangement of λL60 in Linv (Figure 4A), an aberrant rearrangement has taken place on this allele. An interesting feature of both the non-productive rearrangement in Pt and in Linv is that they both occur very close to the 5′ end of J_α segments at chromosome band 14q11, which is where normal TCR V–J_α rearrangements would occur.

The non-productive rearrangements correspond to the 14q32 breakpoints of the inv(14) and t(14;14)

The inability to identify the sequences adjacent to the non-

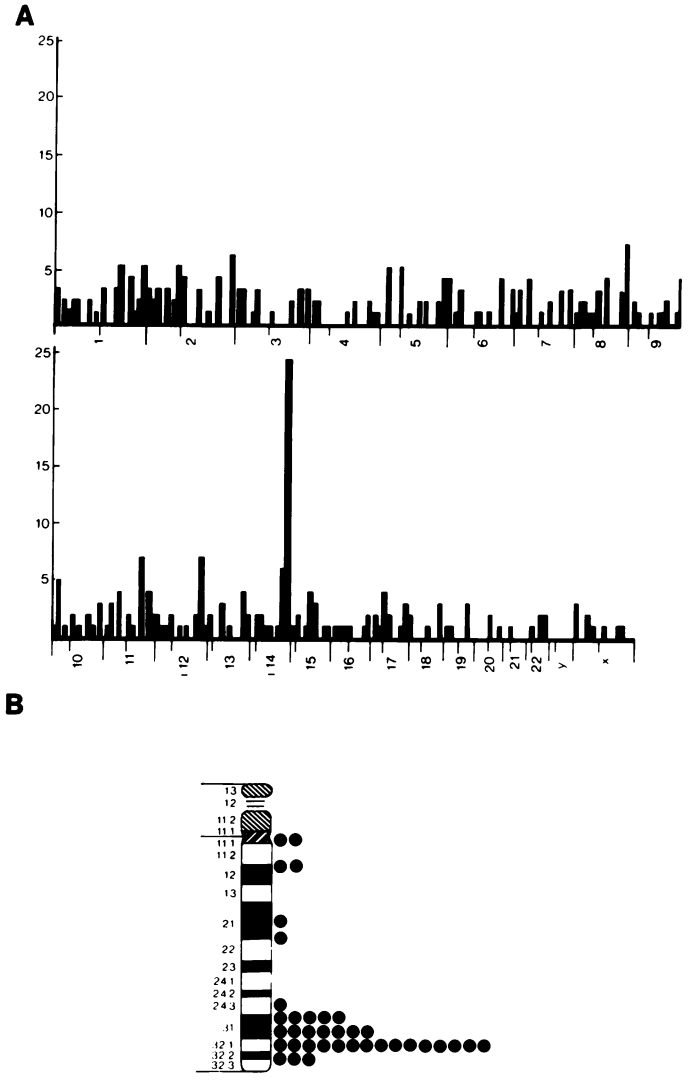

Fig. 6. *In situ* hybridization of Linv breakpoint probe. Metaphase chromosomes were hybridized to ^{3}H-labelled pL60.HH.18 and silver grains counted over G-banded chromosomes. **(A)** Histogram of 369 grains counted from 64 metaphases. **(B)** Distribution of specific grains over chromosome 14.

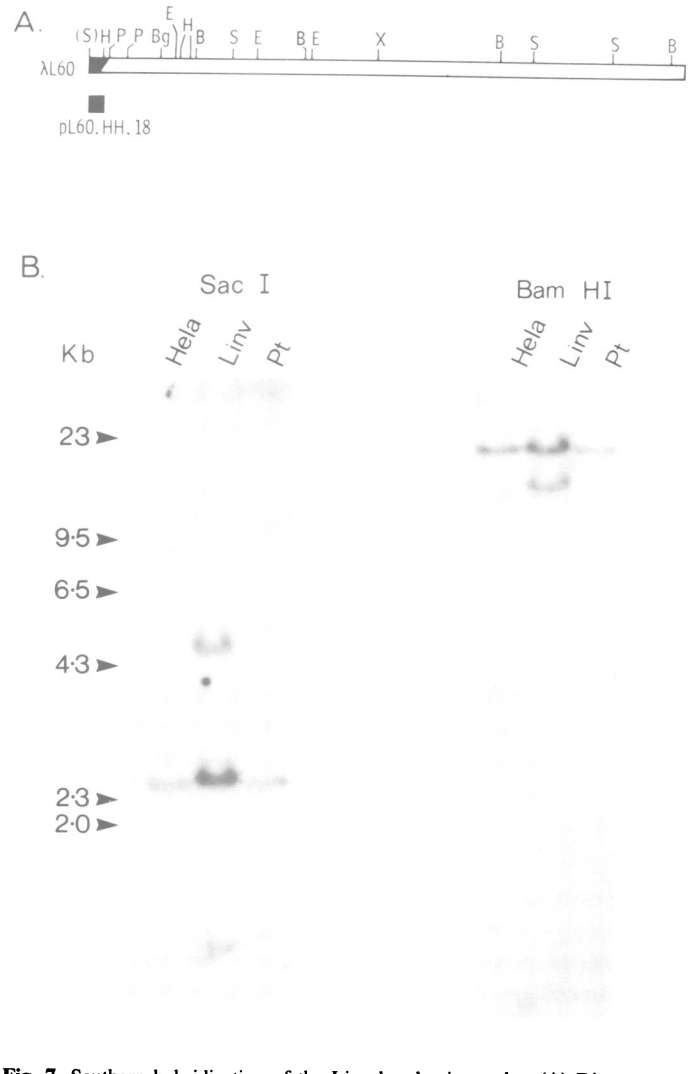

Fig. 7. Southern hybridization of the Linv breakpoint probe. **(A)** Diagram of pL60.HH.18 and map of λL60 from which the probe was derived. **(B)** Ten micrograms of HeLa, Linv or Pt DNA were digested to completion with the enzymes indicated, electrophoresed and transferred to cellulose nitrate filters. The filters were hybridized to pL60.HH.18. Sizes are estimated from λ DNA cut with *Hin*dIII.

productive rearrangements in Pt and Linv suggested the possibility that these sequences might derive from chromosome band 14q32, i.e. that they might come from either the translocation t(14;14) or inv(14) breakpoints. This possibility was examined by using DNA prepared from rodent–human somatic cell hybrids carrying translocated chromosomes involving 14q32. Three hybrids were used and the relevant chromosomal content (determined by cytogenetic analysis) is diagrammatically shown in Figure 5E. W4-1A possesses, as well as normal chromosome 14, a t(X;14) chromosome in which 14 pter → 14q32 is present. The translocation partner of this clone (W4-3Aaz2) contains only the reciprocal of the t(X;14), i.e. 14q32 → qter. An unrelated hybrid (W53-5B clone 5) contains a different t(X;14) chromosome with 14pter → 14q24. DNA from these cells was hybridized to either a Ig H-chain J_H probe or to a TCR J_α probe (J_αBX). The results, shown in Figure 5A and B respectively, confirm the cytogenetic assignment. W4-1A and W4-3Aaz2 but not W53-5BC1.5 contain Ig J_H segments whereas W53-5BC1.5 and W4-1A but not W4-3Aaz2 contain J_α segments.

The probes derived from the non-productive J_α alleles described above were hybridized to the DNA of the cell panel. A probe from Linv (pL60.HH.18, Figure 5C) near the end of λL60, hybridizes to a 2.7-kb fragment in normal human DNA. A similar hybridization was only observed in W4-1A; the other two hybrids do not contain hybridizing segments. Since W53-5c15 contains TCR J_α sequences (Figure 5B), and the DNA from this hybrid does not hybridize to pL60.HH.18, it indicates that this probe originates from 14q24 → ter. Conversely, the lack of hybridization to W4-3Aaz2 (which contains Ig J_H sequences, Figure 5A) indicates that pL60.HH.18 comes from proximal to J_H at 14q32. This probe must, therefore, lie between 14q24 and 14q32. Since the cytogenetically defined breakpoints in the Linv T-CLL tumour cells are 14q11 and 14q32, it seems reasonable to conclude that the pL60.HH.18 probe (which was cloned from a position adjacent to TCR J_α at 14q11) comes from chromosome 14q32.

The localization of the Linv breakpoint to 14q32 was confirmed independently by *in situ* hybridization. The pL60.HH.18 clone was hybridized to metaphase chromosomes of normal human lymphocytes and the grain distribution determined. Figure 6A shows a histogram of grains counted over all the human chromosomes. A major peak of hybridization is found only near the long

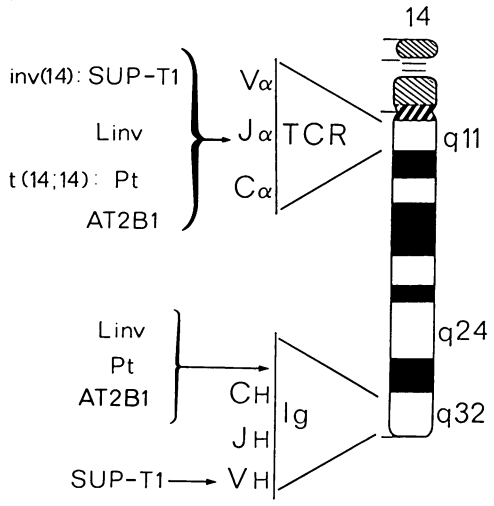

Fig. 8. Diagrammatic representation of chromosome 14 with associated locations of breakpoints. Two tumours with inv(14) are shown (SUP-T1, Linv) and one tumour (Pt) with translocation t(14;14). AT2B1 is a non-leukemic T-cell clone, from an A-T patient, which carries a t(14;14), Kennaugh (1986).

arm of chromosome 14. Figure 6B shows the distribution of silver grains over the chromosome 14 from 64 cells counted. The peak of hybridization occurs at the tip of the long arm of chromosome 14 and corresponds to approximately band q32. However, the data from the somatic cell hybrids indicate that the probe, although from 14q32, is from DNA proximal to Ig J_H at q32. Therefore, the breakpoint is different from the previously described inv(14) breakpoint in the SUP-T1 T cell lymphoma cell line which occurs within the Ig V_H segments, distal to J_H.

A Pt DNA probe from adjacent to the J_α segment of the non-productive allele of this tumour showed a similar chromosomal localization as the Linv non-productive allele. A subclone from λP39 (pBX.SH.24), comprising both TCR J_α sequences and sequences from the non-productive rearranged segment of Pt DNA, was used in filter hybridization with the somatic cell panel (Figure 5D). The probe detects two fragments in *Sac*I-digested unrearranged human DNA, a 5.2-kb fragment derived from the germline TCR J_α locus and a 3.5-kb fragment from the locus corresponding to the non-productively rearranged DNA segment. Both of these fragments are present in W4-1A DNA but neither are present in W4-3Aaz2. On the other hand, the fragment from the J_α locus (5.2 kb) was observed in the W53-5B c1.5 DNA but the 3.5-kb fragment was not. Thus the DNA from the Pt probe which detects the 3.5-kb fragment must lie between 14q24 and 14q32 and, like the Linv probe, be proximal to the immunoglobulin J_H locus (since the J_H probe does hybridize with W4-3Aaz2 DNA). In view of the fact that cytogenetics defines the translocation t(14;14) breakpoints to be bands q11 and q32, it is reasonable to assume that the non-productively rearranged Pt DNA segment also comes from 14q32 since it is directly adjacent to sequences from 14q11 in λP39.

We can conclude from these data that the inv(14) tumour (Linv) and the translocation t(14;14) tumour (Pt) both have non-productive rearrangements within the TCR J_α locus (exemplified in λL60 and λP39 respectively, see Figure 2) which correspond to the breakpoints of the inversion or translocation. Furthermore, both breakpoints involve DNA segments from 14q32, on the centromeric side of the Ig J_H locus. However, Southern filter hybridization experiments show that the two breakpoints are not immediately adjacent to each other at 14q32. Figure 7 shows the results of a hybridization experiment using pL60.HH.18 (Figure 7a) and comparing Linv with Pt DNA. This probe detects a band of ~20 kb in *Bam*HI-cleaved control DNA (HeLa), in Pt and in Linv DNA (Figure 7B). In addition, a 15-kb rearranged fragment is observed in Linv DNA representing the 14q32 breakpoint in this tumour. No rearranged band is seen in Pt DNA. Therefore, the Linv and Pt breakpoints do not coincide in the 20-kb germline *Bam*HI fragment. Similarly, the probe detects unrearranged and rearranged bands in *Sac*I-digested Linv DNA but only an unrearranged band in Pt DNA. Thus, if the breakpoints at 14q32 are in any way clustered, they are not immediately adjacent to each other.

Discussion

inv(14) and t(14;14) T-cell tumours have breakpoints immediately adjacent to TCR J_α segments at 14q11

Both of the tumour biopsy samples examined in this report have abnormalities of chromosome 14 in which breakpoints occur at 14q11 and 14q32. We have shown that these involve the TCRα locus at 14q11 and a segment on the centromeric side of the Ig J_H locus at 14q32. The location of the q11 breakpoints in the tumours is of interest since both occur within the large TCR J_α locus. In addition, rearrangements are precisely mapped to the 5′ ends of different J_α segments in positions homologous to where V_α segments would normally join (e.g. like the productive V_α–J_α rearrangements seen on the other allele in these tumours). This is similar to the previously described inv(14) in the cell line SUP-T1, in which both telomeric (Baer *et al.*, 1985; Denny *et al.*, 1986a) and centromeric breakpoints (Baer *et al.*, 1987) involve TCR J_α segments. However, Linv and Pt 14q32 breakpoints are markedly different from the Ig V_H–TCR J_α fusion gene described near the telomere of chromosome 14 in SUP-T1. In the present cases, no Ig-like sequences are detectable at the breakpoint of the inversion or translocation but rather we find involvement of DNA segments from proximal to Ig J_H at 14q32. Furthermore, it should be noted that both inversion and translocation breakpoints are associated with the allelically excluded (non-productive) allele of TCRα. The possibility that the breakpoints occur within the immunoglobulin heavy chain constant (C_H) region locus has been investigated by hybridization studies with the Pt breakpoint probe and cosmids containing >100 kb of C_H genomic DNA (Flanagan and Rabbitts, 1982a); however no hybridization was found (unpublished). Two gaps of unknown size exist in this cosmid set, however, so we cannot be certain that the breakpoints are fully proximal to the Ig C_H region.

The mechanism of these rearrangements is not clear. However, the translocation t(14;14) in Pt cells may not have been a direct translocation between 14q32 and the J_α locus at 14q11, since the J_α sequences close to, but upstream from, the junction of the translocation are deleted in both alleles of chromosome 14. This may indicate a non-reciprocal translocation or it may imply that secondary rearrangements occurred at the translocation junction resulting in deletion of segments of the J_α locus. This would not be unprecedented in chromosome 14 abnormalities as such a series of events appears to have occurred in the creation of the SUP-T1 inv(14) chromosome (Baer *et al.*, 1987). If this was also the case for Pt, it is possible that the translocation was mediated via sequences proximal to Ig J_H which are homologous to the Ig V_H locus.

No common breakpoint site at 14q32 in T-cell tumours

There have been molecular studies on two inv(14) and one translocation t(14;14) in T-cell tumours to date (Baer *et al.*, 1985;

Denny *et al.*, 1986a; this paper) and diagrammatic summary of the various breakpoints on chromosome 14 is shown in Figure 8. Clearly, there is no obvious clustering of 14q32 breakpoints in cells carrying these abnormalities. One of these [the inv(14) of SUP-T1] has a telomeric breakpoint in the V_H locus. The two cases reported in this paper have telomeric breakpoints on the centromeric side of J_H. A further example of translocation t(14;14) in a T-cell clone (not a tumour) from an A-T patient (Kennaugh *et al.*, 1986) also has a breakpoint proximal to J_H. All of these cases break within TCR J_α at their centromeric sequences. Further, a B-cell tumour with inv(14) breaking in TCRα and Igh has been recently described (Denny *et al.*, 1986b). The analysis of possible relationships between these and other inv(14) or t(14;14) tumours will perhaps rationalize these apparently disparate observations.

Significance of the 14q32 sequences in T-cell tumours

The frequency of occurrence of 14q32 abnormalities in T-cell tumours is a measure of the importance of this chromosome band to tumour aetiology. The inv(14) abnormality has been observed in a high proportion of T-CLL (Zech *et al.*, 1984) and T-PLL (Brito-Babapulle *et al.*, 1987) patients. This occurs at a considerably higher frequency than the appearance of inv(14) in normal human T cells stimulated to divide in culture (Aurias *et al.*, 1985). Thus a considerable selection occurs for this marker chromosome in T-cell tumours of this type, providing evidence for involvement in tumour aetiology. It is possible that the inv(14) abnormality in the SUP-T1 cell line is representative of the type of abnormality found in normal cultured lymphocytes and, therefore, that the proximal breakpoints (e.g. Linv and Pt described here) are the crucial ones for tumorigenesis. The SUP-T1 cell line does carry an abnormality of chromosome 8q24 just downstream of the c-*myc* gene (Rabbitts *et al.*, 1986) which might in itself be important for this specific tumour. Conversely, it may be that the proximal 14q32 breakpoints are not important but the distal position may be crucial for T-cell tumorigenesis. Perhaps more likely is that the two breakpoint areas are, in some way not yet understood, related and that rearrangements at either position can be a contributing factor to the development of T-cell leukaemia. This might be analogous to the situation in Burkitt's lymphoma where there appear to be at least two clusters of segments near the c-*myc* oncogene (reviewed by Klein and Klein, 1985), one of which, the pvt-like region, is at least 200 kb from c-*myc* (Graham and Adams, 1986; Mengle-Gaw and Rabbitts, 1987). The various 14q32 breakpoints will need to be analysed more extensively with respect to each other to allow clarification of their possible functional inter-relatedness.

Materials and methods

Tumour biopsies

DNA was extracted from peripheral lymphocytes of patients Linv and Pt. Karyotypic data was obtained from PHA-stimulated cells. The pertinent features are inv(14) (q11,q32) in Linv and translocation (t14;14) (q11;q32) in Pt cells. Linv has previously been described (patient 6) (Zech *et al.*, 1986).

λ *library preparation and analysis*

λ phage libraries were prepared from size-selected *Sau*3A partial genomic DNA fragments (15–20 kb) cloned in the *Bam*HI site of λ2001 (Karn *et al.*, 1984). Subclones of λ phage clones were prepared in pUC or M13 vectors (Vieira and Messing, 1982). DNA sequence analysis was carried out using dideoxy chain termination methods (Sanger *et al.*, 1977) in M13 vectors, either of specific restriction enzyme fragments or by shot-gun procedures (Bankier and Barrell, 1983; Staden, 1986).

Hybridization procedure

Southern filter hybridization (Southern, 1975) was carried out as previously described (Lefranc *et al.*, 1986) using nick-translated probes (Rigby *et al.*, 1977). Hamster–human cell hybrids have previously described (Willard and Holmes, 1984) and were prepared from GM74 cell line carrying t(X;14) translocation (Camden strains repository). W53-5Bc15 was described by Willard *et al.*, 1985.

In situ hybridization was carried out as described (Buckle and Craig, 1986) using ^{3}H-labelled, nick-translated probes.

Acknowledgements

L.M.-G. is a recipient of a Helen Hay Whitney Fellowship.

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Received on March 16, 1987; revised on April 27, 1987