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A new gene, BCM, on chromosome 16 is fused to the interleukin 2 gene by a t(4;16)(q26;p13) translocation in a malignant T cell lymphoma

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A t(4;16)(q26;p13.1) chromosome translocation found in tumour cells from a patient with a T cell lymphoma was shown to rearrange the interleukin 2 gene, normally located on chromosome band 4q26, with sequences from chromosome band 16p13.1. A cDNA library of tumour cells was screened with an interleukin 2 gene-specific probe. Three clones were isolated, which consisted, from 5′ to 3′, of the three first exons of the interleukin 2 gene followed by a 16p13 in-frame sequence encoding 181 amino acids. A probe derived from this sequence detected a 1.2 kb transcript in various cell lines exhibiting mature B lymphoid cell features, but this was not detected in other cell lines representative of other haematopoietic lineages, or in other organs. For this reason, the novel gene was termed BCM for B cell maturation. The open reading frame of BCM normal cDNA predicted a 184 amino acid protein with a single transmembrane domain which had no homology with any protein sequence stored in data banks. Our data indicate that BCM is a new gene whose expression coincides with B cell terminal maturation.

Key words: chromosome 16/IL2/T cell lymphoma/translocation

Introduction

Molecular analysis of chromosome abnormalities occurring in human lymphoid neoplasias shows that they fall into two main categories. The first juxtaposes immunoglobulin (Ig) or T cell receptor (TcR) loci to a gene from the partner chromosome involved in the translocation. The most documented examples for this type of translocation are the t(8;14) translocation, present in Burkitt's lymphoma (Zech *et al.*, 1976; Dalla Favera *et al.*, 1982; Erikson *et al.*, 1982), and the t(14;18) translocation found in follicular lymphoma (Fukuhara *et al.*, 1979; Yunis *et al.*, 1982; Bakhshi *et al.*, 1985; Cleary and Sklar, 1985; Tsujimoto *et al.*, 1985), which juxtapose the proto-oncogenes c-*myc* and BCL-2, on chromosomes 8 and 18 respectively, to the Ig heavy chain locus on chromosome 14, resulting in the activation of these proto-oncogenes. In the second category of translocations, the chromosome breakpoints split the genes on both partner chromosomes, and lead to juxtaposition of part of each gene in the joint segments and the creation of a composite gene. Because the breakpoints disrupt each gene within an intron, coding sequences of each gene are maintained in the same reading frame and the resulting chimeric transcripts usually encode hybrid proteins. This situation is well illustrated by the t(9;22) translocation, found in acute lymphoblastic leukaemia (ALL), in which a BCR–ABL fusion gene is created (Hermans *et al.*, 1987) and by the t(1;19)(q23;p13.3) translocation, present in pre-B cell ALL, which results in a chimeric gene containing the homeodomain of PBX and the BHLH/leucine zipper domain of E2A encoding an Ig enhancer-binding protein (Kamps *et al.*, 1990; Nourse *et al.*, 1990).

A number of products of the genes activated by these translocations contain structural motifs of transcription regulatory factors, which are important for growth and/or differentiation of haematopoietic cells (Rabbits, 1991; Sawyers *et al.*, 1991; Solomon *et al.*, 1991). There are also some notable exceptions. For instance, the bcl-2 protein is associated with the inner mitochondrial membrane and increasing evidence shows that its constitutive expression in lymphoma cells bearing the translocation confers on these cells the ability to survive *in vivo* for long periods of time (Hockenbery *et al.*, 1990; Sentman *et al.*, 1991; Strasser *et al.*, 1991). In contrast, genes encoding lymphokines have rarely been implicated in chromosome translocations. Only the interleukin 3 gene is known to be involved in a t(5;14)(q31;q32) translocation, found in ALL of B cell lineage with eosinophilia (Grimaldi and Meeker, 1989; Meeker *et al.*, 1990), supporting the idea that inappropriate expression of a growth factor may also be an element of certain leukaemogenic processes.

Here, we report the molecular analysis of a t(4;16)(q26;p13) translocation, found in tumour cells of a patient with an intestinal T cell lymphoma. We have shown that the interleukin 2 (IL2) gene, normally located on chromosome 4q26 band, was rearranged. Upon cloning of the rearranged IL2 fragments, we have established the nucleotide sequence of the chromosome breakpoints and that of the normal counterparts of chromosome band 16p13. In addition, the presence in a tumour cell cDNA library of a hybrid cDNA resulting from the fusion of the IL2 gene with novel coding sequences of chromosome band 16p13, provided evidence for a new transcriptional unit located on this chromosome band.

Results

Patient BEL was initially admitted for a malabsorption syndrome. An intestinal $CD3^+$, $CD4^+$, $\beta1F^+$ T cell lymphoma was thereafter diagnosed. Chromosome examination of peripheral blood leukocytes (PBL) from the patient showed an abnormal karyotype: 46,XY,t(4;16)(q26;p13), present in 11 metaphases, while 12 metaphases exhibited a normal karyotype (Figure 1). The translocation, thus, resulted in 4q– and 16p+ derivative chromosomes.

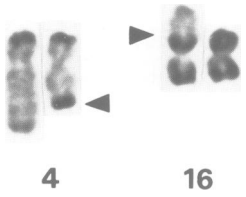

Fig. 1. t(4;16)(q26;p13). Partial R-banded karyotype. This figure shows the abnormal chromosomes 4 and 16 in the malignant cells of patient BEL. Abnormal $4q^-$ and $16p^+$ chromosomes are indicated by arrows.

IL2 gene is rearranged in BEL malignant cells

Since the IL2 gene has been localized on 4q26-q28 chromosome bands (Seigel *et al.*, 1984; Sykora *et al.*, 1984), the possibility that it was close to the chromosome breakpoint of the t(4;16)(q26;p13) translocation was investigated. DNA was isolated from lymph nodes of patient BEL, analysed to determine the configuration of the IL2 gene, and compared with that of human placental DNA. As shown in Figure 2, an IL2 cDNA probe revealed two rearranged *Eco*RI fragments of 4.5 and 6.5 kb, in addition to the 3.8 kb normal germ-line band. Digestion with other restriction enzymes also showed the presence of rearranged bands: two 23 and 19 kb rearranged *Bam*HI bands; one rearranged *Hin*dIII band (>30 kb) and two 15 and 4.2 kb rearranged *Pst*I bands. These data excluded the possibility of a restriction fragment length polymorphism and indicated that the IL2 gene of the malignant cells of patient BEL had been modified by the translocation.

Molecular cloning of IL2 rearranged genomic fragments

Two partial genomic libraries were constructed from *Eco*RI-digested lymph node BEL DNA in λgt10 *Eco*RI-digested phage arms and screened with the IL2 cDNA probe. Two recombinant phages, containing the 4.5 and 6.5 kb rearranged IL2 fragments respectively were isolated. Their partial restriction maps are shown in Figure 3A and C respectively. Comparison of these maps with that of a normal IL2 fragment (Figure 3B) revealed that the breakpoint (arrowed in Figure 3B) on chromosome 4 was located between the third and fourth exons of the IL2 gene, in a region flanked by two *Xba*I restriction sites. We also noticed that the *Hin*dIII restriction site, boxed in Figure 3B, had disappeared. A more accurate description of the translocation breakpoint was established by nucleotide sequencing of breakpoint regions of both rearranged fragments (Figure 4). Comparison of these sequences with that of the normal IL2 gene showed that the breakpoint on the 4.5 kb fragment (Figure 4A) was located 261 bp 3′ of the *Hin*dIII restriction site (boxed in Figure 3B). On the 6.5 kb fragment (Figure 4C), the breakpoint was located 44 bp 5′ of an *Xba*I restriction site. In addition, this comparison indicated that the translocation resulted in a 20 bp deletion in the intron between the third and the fourth exons of the IL2 gene. Moreover the sequence data indicated that the disappearance of the *Hin*dIII site boxed in Figure 3B resulted from a point mutation (data not shown). From these data, the 4.5 and 6.5 kb fragments appeared to represent the derivative partners of the t(4;16)(q26;p13) translocation.

Comparison of the sequences juxtaposed to the IL2

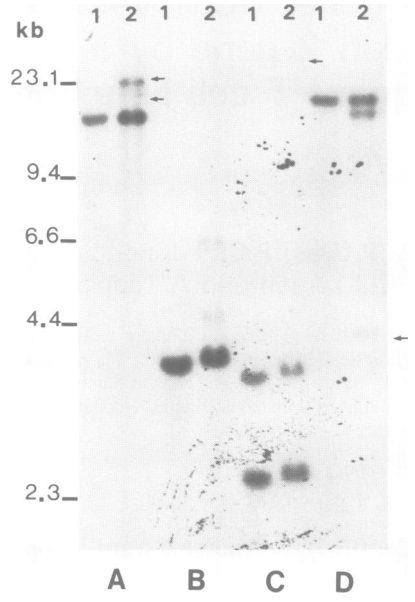

Fig. 2. Southern blot analysis of lymph node BEL DNA. Ten microgrammes of normal human placenta (1) and/or lymph node BEL DNA (2) were digested by *Bam*HI (**A**), *Eco*RI (**B**), *Hin*dIII (**C**) and *Pst*I (**D**) restriction enzymes, electrophoresed on a 0.7% agarose gel and transferred to nylon membrane filter. The filter was hybridized using a cDNA IL2 probe, as described in Materials and methods. Arrows indicate the faint rearranged bands.

sequences by the translocation showed no homology with any sequence stored in EMBL and/or GenBank data banks.

IL2 gene expression in BEL malignant cells

In order to determine whether the t(4;16) translocation affected the expression of the IL2 gene, we looked for the presence of IL2 transcripts in poly(A)$^+$ mRNA extracted from BEL lymph nodes and total mRNA from BEL PBLs before and after 16 h stimulation with phytohaemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) PHA + PMA (Nowell, 1960; Yamamoto *et al.*, 1985). IL2 expression was also examined in PHA + PMA-stimulated normal PBLs, taken as a control. The results are presented in Figure 5. While a normal IL2 transcript, with an expected size of ~850 nt was detected in the lane corresponding to stimulated normal PBL RNA, a faint signal of normal length was detected in the stimulated BEL PBL cell lane. There was no signal of normal and/or abnormal length in the unstimulated BEL PBL cell lane. Hybridization of the filter with a probe specific for the actin gene ascertained that the absence of an IL2 signal was not due to RNA degradation.

Cloning of a hybrid cDNA, containing IL2 sequences

In spite of the absence of any IL2 mRNA detected by the Northern blot analysis, we constructed a cDNA library from poly(A)$^+$ mRNA of BEL lymph nodes. Subsequent screening of 5×10^5 recombinant λgt10 phages of this library with the IL2 cDNA probe resulted in the isolation of three recombinant phages. The nucleotide sequence of these clones showed that they were cDNA clones containing normal IL2 sequences in their 5′ part (Figure 6). Homology with IL2 was abruptly interrupted (for all three clones) at the end of the third IL2 exon. The novel sequences were in-frame with the IL2 sequences and had the capacity to encode 181 amino acids. Since all clones appeared to contain

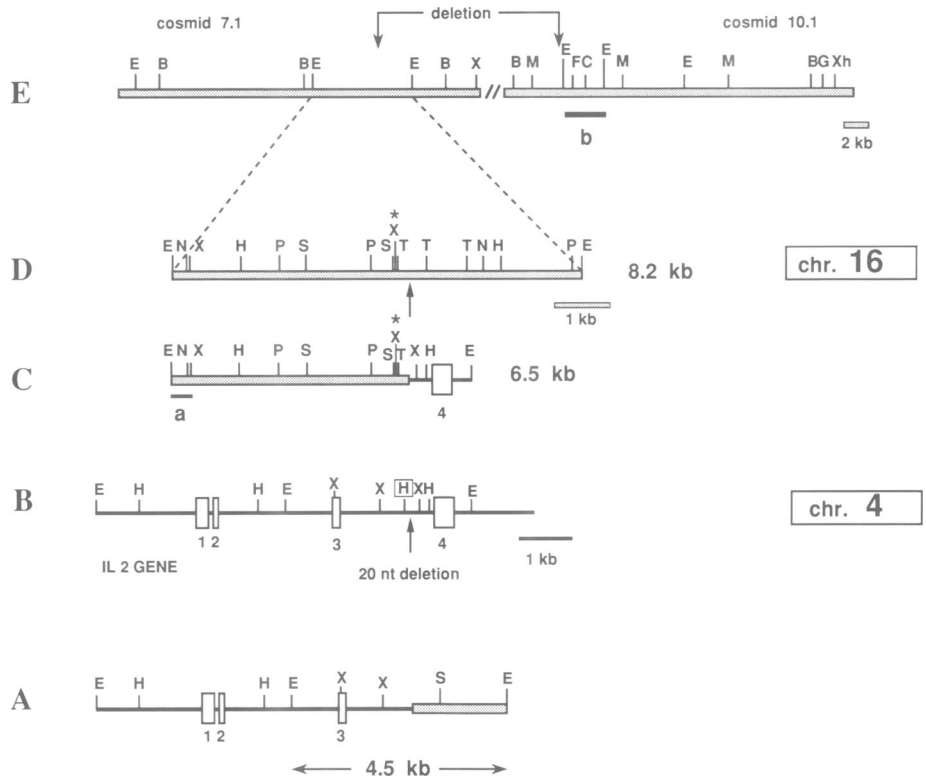

Fig. 3. Partial restriction maps of rearranged genomic IL2 fragments and of their normal counterparts on chromosome bands 4q26 and 16p13.1. The partial restriction maps of rearranged IL2 fragments are represented in (**A**) for the 4.5 kb fragment and in (**C**) for the 6.5 kb fragment. The restriction map of the normal IL2 gene (Degrave *et al.*, 1983; Fujita *et al.*, 1983) is shown in (**B**), while the germ-line 8.2 kb counterpart of chromosome band 16p13 is shown in (**D**). This region was cloned using the 0.4 kb *Eco*RI−*Xba*I (probe a). (**E**) presents the restriction maps of cosmids 7-1 and 10-1 of chromosome band 16p13 cloned using probes a and c respectively. Chromosome 4 sequences are depicted by a thick line, those of chromosome 16 are by a grey line. The boxed *Hin*dIII restriction site (B) is the one that is missing (because of a point mutation) on the 4.5 kb rearranged IL2 fragment (A). Arrows show the translocation breakpoints. The 2.7 kb *Eco*RI genomic fragment hybridizing with probe c is underlined in (E) and annotated as b. Restriction enzymes: E, *Eco*RI; B, *Bam*HI; X, *Xba*I; F, *Sfi*I; M, *Sma*I; H, *Hin*dIII; C, *Cla*I; G, *Bgl*II; Xh, *Xho*I; P, *Pst*I; N, *Nco*I; S, *Sph*I; and T, *Stu*I.

the first three exons of IL2 and novel sequences at their 3′ end, they were likely to originate from the rearranged IL2 gene fused with sequences from chromosome band 16p13. It is worth noting that a 0.7 kb *Sph*I−*Eco*RI (probe c in Figure 6) fragment, containing these putative 16p13 sequences, again did not produce any signal on Northern blotting of BEL cell mRNA (data not shown).

There was no homology between the putative 16p13 novel sequences with any sequences stored in GenBank and/or EMBL data banks. A negative result was also obtained after search for a known protein motif in the PROSITE data bank.

The novel sequence in the hybrid IL2 cDNA maps to the 16p13 chromosome band

The aforementioned 0.7 kb *Sph*I−*Eco*RI fragment (probe c) was used to carry out *in situ* hybridization. A total of 293 metaphases were examined in three independent experiments. Forty-five out of 299 grains (15%) were localized on chromosome 16, and 33 (11% of the total number of grains and 73.3% of those on chromosome 16) were on band 16p13.1. From these results, we concluded that the cDNA hybrid clones were transcribed from a fusion gene generated by the t(4;16)(q26;p13) translocation.

Cloning of the normal 16p13 counterpart fragments

In order to isolate the normal germ-line 16p13 DNA region, containing the translocation breakpoint, we prepared probes representative of the sequences juxtaposed to IL2 sequences in the rearranged fragments. A preliminary analysis showed that only probe a (underlined in Figure 3C), was devoid of repetitive sequences and could be used for cloning.

Probe a revealed a unique 8.2 kb *Eco*RI band in normal placental DNA. Therefore, it was used to isolate this 8.2 kb *Eco*RI fragment from a partial genomic BEL DNA library constructed in λZAPII *Eco*RI phage arms (see Materials and methods). Three identical phage clones were obtained, whose restriction maps are presented in Figure 3D. A comparison of the restriction map of this fragment with those of both rearranged IL2 fragments showed that its 5′ end was identical to the non-IL2 sequences translocated to the fourth IL2 exon in the 6.5 kb rearranged fragment. In contrast, the 3′ end differed from the non-IL2 sequences translocated to the three first IL2 exons, in the 4.5 kb rearranged fragment. The nucleotide sequence located 3′ to the *Xba*I site (marked by an asterisk in Figure 3D) is presented in Figure 4D. The comparison of this sequence with those of rearranged IL2 fragments showed that it is identical in its 5′ part to the non-IL2 sequence juxtaposed to the fourth exon of the IL2 gene in the 6.5 kb fragment, while it is completely different from that juxtaposed to the three first exons of IL2 in the 4.5 kb rearranged fragment. One simple but not exclusive hypothesis to explain the discrepancy between the non-IL2 sequences juxtaposed to the IL2 gene and those cloned in the normal 8.2 kb fragment is that the translocation was

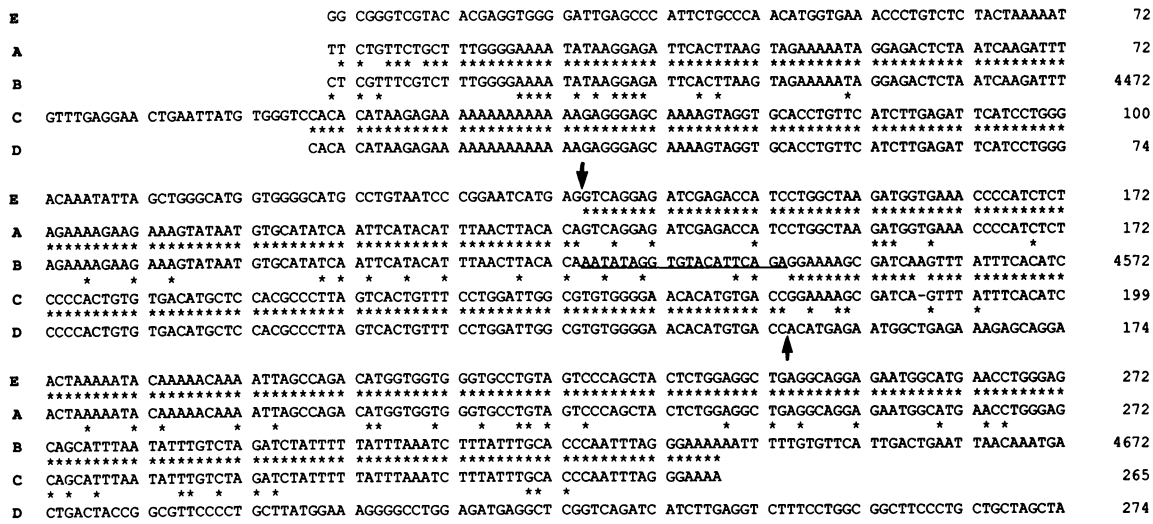

Fig. 4. Comparison of the nucleotide sequences of the translocation breakpoints with those of their normal counterparts. The nucleotide sequence of the chromosomal breakpoints of rearranged IL2 fragments is presented in line A and line C for the 4.5 and 6.5 kb fragments respectively. The normal sequence (Holbrook *et al.*, 1984) of the corresponding part of the IL2 gene is presented in line B. The 20 bp stretch deleted during the translocation is underlined. Line D shows the nucleotide sequence of the corresponding normal 16p13 chromosome 8.2 kb fragment, while lane E respresents the nucleotide sequence of the normal chromosome band 16p13 cosmid 10-1. Homology is depicted by asterisks. The translocation breakpoints are shown by arrows.

accompanied by a deletion of a DNA segment of chromosome 16, whose size was not less than 4.2 kb (from the *Stu*I site to the 3′ end in Figure 3D).

In order to demonstrate the colinearity of the rearranged sequences from the genomic clones and the cDNA recombinant clone, a normal human placental DNA cosmid library constructed in the *Bam*HI site of the pWE 15 cosmid vector was screened with probe a and probe c (representing 16p13 sequences of the hybrid cDNA). Two distinct cosmid clones hybridizing with probe a (7-1 cosmid) and probe c (10-1 cosmid) were isolated. Comparison of their partial restriction maps shown in Figure 3E showed no overlapping regions. However, fluorescence *in situ* chromosome hybridization indicated that the 7-1 cosmid mapped to chromosome 16p13.1 (data not shown). While these results strongly argued in favour of the colinearity of the sequences of the genomic DNA and of the non-IL2 cDNA sequences, they provided no information about (i) the precise extent of the deletion associated with the translocation, and (ii) the distance separating the 16p13.1 sequences composing the genomic joint from those fused to the three first exons of the IL2 gene. Hybridization experiments followed by establishment of nucleotide sequence of cosmid 10.1 allowed us to localize the sequences of chromosome 16p13 juxtaposed to the three first exons of IL2. Comparison of this sequence with those of rearranged IL2 fragments is represented in Figure 4E. These results allowed a better estimation of the distance separating the 16p13.1 breakpoint from the 16p13.1 coding sequences found in the chimeric cDNA clones. As these coding sequences are contained in fragment b (a 2.7 kb *Eco*RI fragment underlined in Figure 3E) and the breakpoint is located immediately upstream of the *Eco*RI restriction site, we can assume that the distance between them was between 2 and 5 kb. The restriction map of the 4.5 and 6.5 kb fragments also showed that the deletion occurring on chromosome 16p13 was at least 4.2 kb long. The restriction maps of the cosmids isolated and the precise localization of the breakpoint corresponding to the 4.5 kb fragment now gave us the opportunity to calculate that the extent of the deletion associated with the translocation was at least 12 kb long.

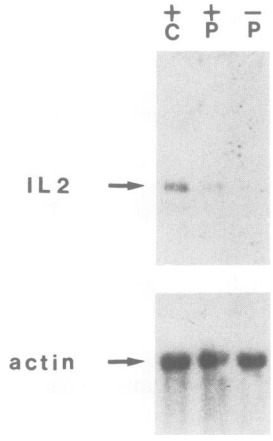

Fig. 5. Northern blot analysis of normal peripheral blood cells (C) and malignant BEL cells (P) was performed before (−) and after (+) stimulation, using an IL2 cDNA probe.

A search for homology between the sequences of chromosome 16 and those stored in GenBank and EMBL data banks was performed. We did not find any homology for the sequences juxtaposed to the fourth exon of IL2 (Figure 4C), while the sequences of chromosome 16 joined to the three first exons of IL2 are highly homologous with a previously described *Alu* sequence (Perlino *et al.*, 1985).

RNA sequences homologous to the 16p13.1 coding sequences are transcribed in B cell lines

To identify the normal cellular lineage that expressed a transcript homologous to the 16p13.1 chromosome band sequences, we hybridized probe c to Northern blots of total RNA from human adult and fetal organs. All the tissues tested, including placenta, brain, lung, spleen, kidney, bladder, skin, muscle and thymus, showed no signal. In

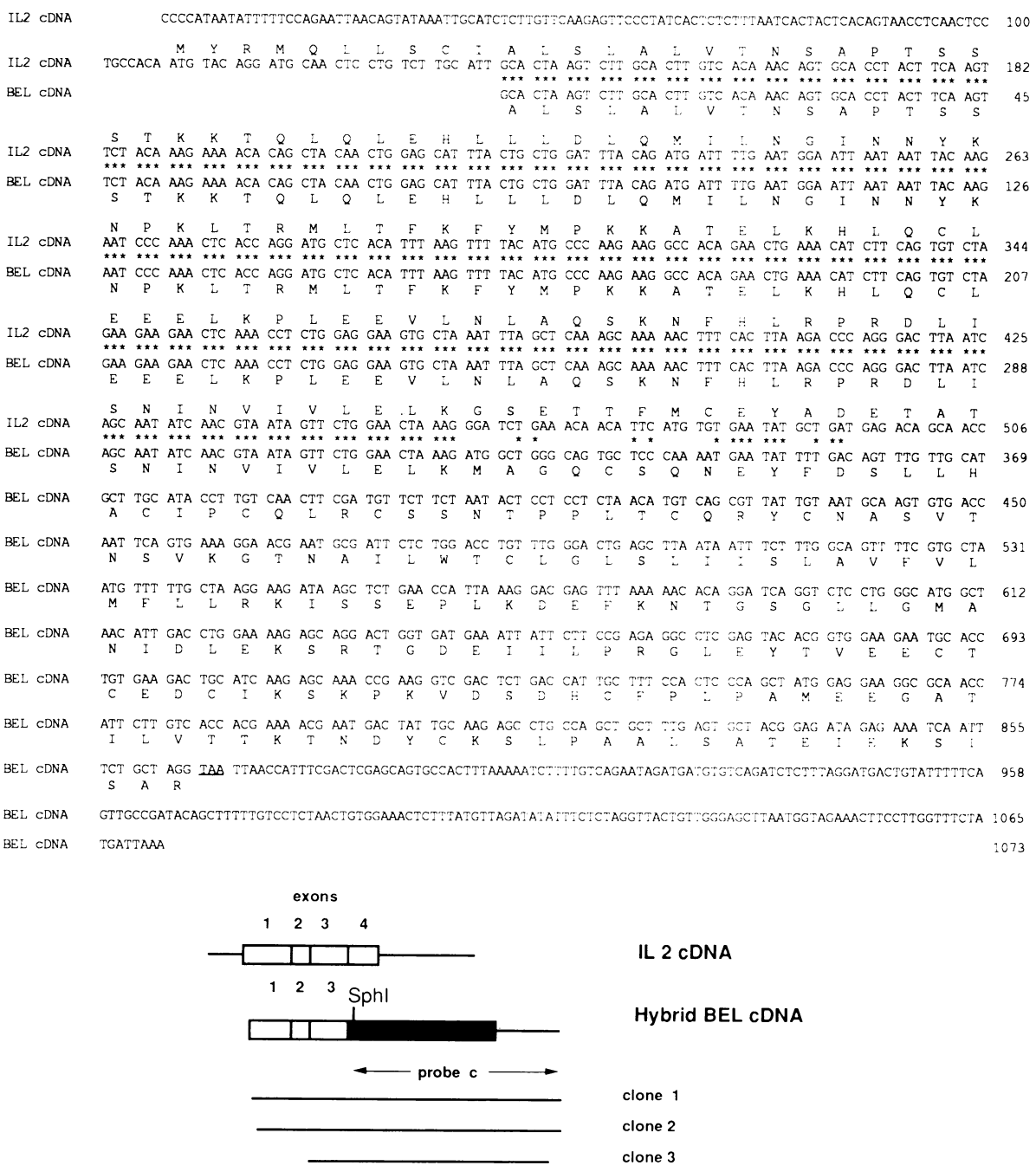

Fig. 6. The nucleotide sequence of BEL hybrid cDNA is compared with that of a normal IL2 cDNA. Numbering of nucleotide residues is that published previously for IL2 cDNA (Holbrook *et al.*, 1984). IL2 cDNA is not presented in totality. Homology is depicted by asterisks and is 100% until residue 458 for IL2 cDNA and residue 321 for BEL cDNA. At this point homology ceases abruptly. In-frame sequence continues until residue 864 (BEL cDNA), where there is a stop codon (underlined). A schematic representation of this comparison is presented in the lower part of the figure. IL2 coding sequences are represented by white boxes, while the novel 16p13 coding sequences are represented by black box. Non-coding sequences are shown as lines. The restriction site *Sph*I is shown, as well as the extent of the probe c used further in hybridization and cloning experiments. The extent of the three cDNA clones isolated is also shown in the same figure.

contrast, a 1.2 kb RNA species was revealed in the Burkitt cell line Daudi, in several Epstein–Barr virus (EBV)-transformed normal human lymphoblastoid cell lines, in human plasmocytic cell lines RPMI 8226 and U266, and in pokeweed-stimulated human B cells, while it was lacking in resting human B cells and in human B cells stimulated by anti-μ antibodies (Figure 7). This RNA was not observed in resting T cells or T cells activated with either PHA or PHA + PMA or pokeweed mitogen. Negative results were also obtained with $\alpha\beta$ T cell lines (Jurkat, MOLT-16), $\gamma\delta$ T cell line Peer or $CD3^-$ T cell line CEM. In addition, monoblastic (U937 ± PMA), promyelocytic (HL-60), multipotent haemopoietic cell (K562), pre-B cell (NALM-6, Jea, Reh, KM3), Burkitt's lymphoma Raji, breast cancer cell (HSL53, T47D), neuroblastoma cell (IMR32), human teratocarcinoma cell (Tera 2) and megakaryocytic (MLA) cell lines revealed no signal. These results indicate that the 1.2 kb RNA homologous to probe c is expressed mainly, if not only, in mature B cells. For this reason the corresponding gene was named BCM for B cell maturation.

Cloning of normal BCM cDNA

To characterize the product encoded by the BCM gene further, a cDNA library from poly(A)$^+$ mRNA of an EBV-transformed lymphoblastoid human cell line was constructed and screened with probe c. Eight independent incomplete [poly(A)$^-$] cDNA clones were isolated and their nucleotide sequences established (Figure 8). The DNA sequences of composite cDNA revealed an open reading frame of 552 bp in-frame with the sequence that was fused to the IL2 gene in the IL2−BCM hybrid cDNA. It starts at position 67 with an imperfect Kozak consensus sequence for the initiation codon (Kozak, 1986) and encodes 184 amino acids with a TAA stop codon at position 619. The sequence starting from position 76 is identical to the sequence fused to the IL2 cDNA.

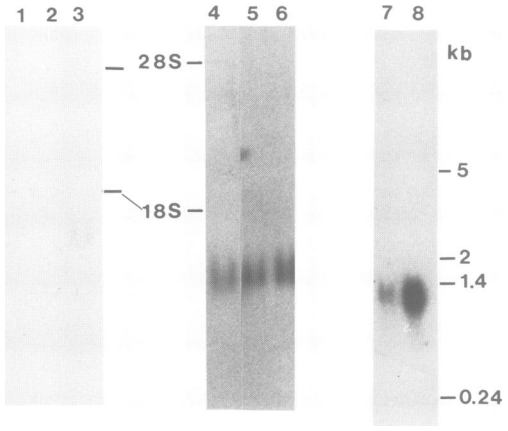

Fig. 7. Northern blot analysis of total mRNA. Total mRNA (10 μg) was electrophoresed in formaldehyde denaturing agarose and transferred to nitrocellulose filter. The filter was hybridized with probe c (Figure 4). Lane 1, normal human spleen B cells; lane 2, normal human B cells after 3 days stimulation by anti-μ antibodies; lane 3, normal human B cells after 7 days stimulation with pokeweed mitogen; lane 4, Daudi cell line; lanes 5 and 6, two EBV-transformed human lymphoblastoid cell lines; lane 7, U266 human cell line; and lane 8, RPMI 8226 human cell line.

The deduced structure of the bcm protein was analysed by different programs. Kyte−Doolittle program analysis (Kyte and Doolittle, 1982) predicted a highly hydrophobic segment of 30 amino acids (residues 52−82). Additional Klein−Kanehisa−DeLisi (Klein *et al.*, 1985) and hydrophobicity moment (Eisenberg, 1984) program analyses strongly suggested that the protein is an integral transmembrane protein. It is noteworthy that no signal peptide cleavage consensus sequence (von Heijne *et al.*, 1986) was found. Further research for known protein patterns using Mac Pattern software (Fuchs, 1991) in the PROSITE database (Bairoch, 1991) revealed a putative site of glycosylation (Marshall, 1972) at Asp42 as well as a possible phosphorylation site by protein kinase C (Kishimoto *et al.*, 1985; Woodgett *et al.*, 1986) at Ser182.

Discussion

In this report, we have analysed a t(4;16) translocation found in tumour cells from a patient with a T cell intestinal lymphoma. We have demonstrated the implication of the IL2 gene on chromosome band 4q26 and a new gene on band 16p13, which encodes a transmembrane protein. Importantly, normal expression of this gene was limited to cell lines as well as to normal B cells corresponding to terminal stages of B cell differentiation (for this reason, it was termed BCM for B cell maturation). Therefore, although this translocation does not currently appear to be recurrent, the nature of the rearranged genes is of interest in view of their established (IL2) or postulated (BCM) role in lymphoid proliferation and/or differentiation processes. In addition, the consequences of this rearrangement, i.e. gain or loss of functions, on the evolutionary process of the tumour should also be considered.

Analysis of rearranged DNA segments issued from each chromosome partner of the translocation showed that the breakpoint on chromosome 4 had interrupted the IL2 gene within intron 3 resulting in the juxtaposition of the three 5′ terminal exons to a DNA sequence from band 16p13. Moreover, alignment of the rearranged sequences with their

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TTGTAAGATATTACTTGTCCTTCCAGGCTGTTCTTTCTGTAGCTCCCTTGTTTTCTTTTTGTGATCATGTTGCAG    75
                                                              M  L  Q      3

ATGGCTGGGCAGTGCTCCCAAAATGAATATTTTGACAGTTTGTTGCATGCTTGCATACCTTGTCAACTTCGATGT   150
 M  A  G  Q  C  S  Q  N  E  Y  F  D  S  L  L  H  A  C  I  P  C  Q  L  R  C     28

TCTTCTAATACTCCTCCTCTAACATGTCAGCGTTATTGTAATGCAAGTGTGACCAATTCAGTGAAAGGAACGAAT   225
 S  S  N  T  P  P  L  T  C  Q  R  Y  C  N  A  S  V  T  N  S  V  K  G  T  N     53

GCGATTCTCTGGACCTGTTTGGGACTGAGCTTAATAATTTCTTTGGCAGTTTTCGTGCTAATGTTTTTGCTAAGG   300
 A  I  L  W  T  C  L  G  L  S  L  I  I  S  L  A  V  F  V  L  M  F  L  L  R     78

AAGATAAGCTCTGAACCATTAAAGGACGAGTTTAAAAACACAGGATCAGGTCTCCTGGGCATGGCTAACATTGAC   375
 K  I  S  S  E  P  L  K  D  E  F  K  N  T  G  S  G  L  L  G  M  A  N  I  D    103

CTGGAAAAGAGCAGGACTGGTGATGAAATTATTCTTCCGAGAGGCCTCGAGTACACGGTGGAAGAATGCACCTGT   450
 L  E  K  S  R  T  G  D  E  I  I  L  P  R  G  L  E  Y  T  V  E  E  C  T  C    128

GAAGACTGCATCAAGAGCAAACCGAAGGTCGACTCTGACCATTGCTTTCCACTCCCAGCTATGGAGGAAGGCGCA   525
 E  D  C  I  K  S  K  P  K  V  D  S  D  H  C  F  P  L  P  A  M  E  E  G  A    153

ACCATTCTTGTCACCACGAAAACGAATGACTATTGCAAGAGCCTGCCAGCTGCTTTGAGTGCTACGGAGATAGAG   600
 T  I  L  V  T  T  K  T  N  D  Y  C  K  S  L  P  A  A  L  S  A  T  E  I  E    178

AAATCAATTTCTGCTAGGTAATTAACCATTTCGACTCGAGCAGTGCCACTTTAAAAATCTTTTGTCAGAATAGAT   675
 K  S  I  S  A  R                                                             184

GATGTGTCAGATCTCTTTAGGATGACTGTATTTTTCAGTTGCCGATACAGCTTTTTGTCCTCTAACTGTGGAAAC   750
TCTTTATGTTAGATATATTTCTCTAGGTTACTGTTGGGAGCTTAATGGTAGAAACTTCCTTGGTTTCTATGATTA   825
AAGTCTTTT                                                                     834
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Fig. 8. Nucleotide sequence of normal BCM cDNA. The numbering of nucleotides is given on the right of the figure. The numbering of amino acid sequence starts at position 67 and stops at position 619, where the TAA stop codon is underlined.

respective germline counterparts revealed deletions on both derivative chromosomes. A 20 bp fragment corresponding to part of intron 3 of the IL2 gene and at least 12 kb of sequences from chromosomal band 16p13 had been deleted in the rearranged fragments. Examination of the germ-line sequences spanning the breakpoints failed to reveal the presence of any particular element such as recombination-specific signals (RSSs), which are recognized by the recombinase complex and have been invoked to explain faulty gene rearrangements involving Ig and/or TcR genes. On the other hand, *Alu* repeats, which have been previously mentioned as possible hot-spots for recombination (Calabretta *et al.*, 1982; Lehrman *et al.*, 1985), were detected in the vicinity of one of the breakpoints on chromosome band 16p13. However, the absence of *Alu* repeats in the vicinity of the corresponding breakpoint on chromosome band 4q26, excludes the hypothesis that the translocation was established by recombination of such sequences.

The most interesting aspect of our work was the characterization of cDNA clones containing the first three IL2 gene exons and sequences from band 16p13.1. These 16p13.1 sequences were shown to be colinear with those contained in the genomic rearranged clones of patient BEL, implying that the chimeric cDNA clones were transcribed from the rearranged IL2 gene. Moreover, a probe representative of the 16p13 sequence (probe c) detected an unequivocal signal in total RNA from EBV-induced human lymphoblastoid cells and the Daudi cell line, demonstrating that the translocation had interrupted a transcription unit. The next step consisted in evaluating the expression pattern of this gene in a variety of haemopoietic and non-haemopoietic tissues. A 1.2 kb transcript was present in cell lines exhibiting features of B cell terminal differentiation but not in T cells, in other haemopoietic lineages or in non-haemopoietic tissues. These data led us to conclude that the expression of the new gene is tissue specific.

The predicted amino acid sequence of the BCM gene is that of a transmembrane integral protein. Putative glycosylation and phosphorylation sites are present, depending on the orientation of the protein in the membrane (Hartmann *et al.*, 1989).

Detailed analysis of the chimeric IL2−BCM cDNA isolated from a BEL tumour identified some structural features that may be significant. The IL2 part of the cDNA matches the sequence of the three exons of the IL2 gene. The sequence then diverges, matching exactly that of the BCM cDNA from nucleotide 76. This fusion, which did not disrupt the original reading frame of the gene, is thus compatible with a normal splicing event between IL2 exon 3 and a BCM exon. Indeed, the BCM cDNA sequence preceding the ATG codon at position 76 contains the AG dinucleotide and an upstream T stretch expected for an intron−exon junction. This would remove three upstream codons starting at position ATG.

What could be the consequences of the translocation in the tumour cells of patient BEL? At least three possibilities should be discussed. First, the IL2−BCM chimeric gene may have generated a fusion protein composed of the N-terminal part of the IL2 product and the totality of the sequence encoding BCM, deleted of three N-terminal amino acids. The binding domain of IL2 to its receptor has been located in the N-terminal part of the protein, but a cysteine residue from exon 4 is also needed, which is lacking in the fusion protein (Smith, 1988). In any case, the presence of low amounts of chimeric transcript (since they have not been detected on Northern blots) is not suggestive of a role for the fusion protein, unless the low levels of chimeric RNA reflect its high instability. Examination of the untranslated sequence does not show the presence of consensus sequences that confer instability (Shaw and Kamen, 1986).

The two other possibilities result from the structural impairment of IL2 or BCM genes that could have led to the loss of their function. Since IL2 plays a key role in growth (Smith, 1988) and probably in differentiation of T cells (Plum and De Smedt, 1988; Tentori *et al.*, 1988; Lenardo, 1991), the modifications of its structure as a consequence of the translocation might have contributed to the malignant process in this particular tumour. Consistent with this, no abnormal IL2 message could be detected in BEL tumour RNA. As for the BCM gene, the question of its possible oncogenic power will be hypothetical until we have clear insight into its physiological function.

The existence of a gene involved in oncogenic processes and located on chromosome 16p13, has been suggested by several authors (Le Beau *et al.*, 1983; Larson *et al.*, 1986). In fact, cells from acute myelomonocytic leukaemia with eosinophilia (M4 EO), frequently exhibit a pericentric inversion inv16(p13;q22) (Le Beau *et al.*, 1983; Sandberg, 1990) and more rarely a t(16;16)(p13;q22) translocation. A t(8;16)(p11;p13) translocation has also been reported in acute monocytic leukaemia (M5) (Heim *et al.*, 1987; Laï *et al.*, 1987; Sandberg, 1990). Though it has recently been shown that the inv16(p13;q22) and the t(8;16)(p11;p13) translocation breakpoints occur in two different loci (Wessels *et al.*, 1991), it will be of interest to test whether at least one of these breakpoints might implicate the new gene rearranged by the t(4;16)(q26;p13) translocation.

In conclusion, the finding of the BCM gene that appears to be expressed in mature B cells is of major interest. The study of its structure and its physiological expression are currently in progress.

Materials and methods

Case report of patient BEL

Patient BEL was admitted for chronic intestinal malabsorption syndrome. Histological and immunohistochemical studies revealed the presence of a lymphoproliferative syndrome of mature T cells ($CD3^+$, $CD4^+$, $CD2^+$, $CD5^+$, $CD25^-$, $\beta1F^+$). The monoclonality of this proliferation was ascertained by the presence of a rearranged band of the TcRβ gene (data not shown).

Chromosome studies

Chromosome studies were performed from PHA-stimulated blood cell cultures, and G-banding (Wright's staining) and R-banding techniques were applied. The chromosomes were classified according to international nomenclature (Harnden and Klingen, 1985).

In situ hybridization

In situ hybridization experiments were performed on high-resolution metaphase chromosomes of PHA-stimulated blood cell cultures from normal male patients with their consent. A 0.7 kb *Sph*I−*Eco*RI fragment, which corresponds to the non-IL2 sequences of the hybrid cDNA isolated, was ^{32}P-labelled by hexanucleotide random priming, using a multiprime DNA labelling kit (Amersham, UK), to obtain a specific activity of 6×10^7 c.p.m./μg. The *in situ* hybridization technique was as described elsewhere (Harper and Saunders, 1981; Caubet *et al.*, 1985). G bands were obtained using Wright's staining.

Southern and Northern blot hybridization

All procedures of DNA digestion, agarose gel electrophoresis, Southern blot transfer, random primer probe labelling and hybridization were carried out as previously described (Davis *et al.*, 1986). RNA was isolated from

nitrogen-frozen lymph nodes of patient BEL, using the guanidinium isothiocyanate method (Chirgwin *et al.*, 1979). Total mRNA Northern blots were performed as described elsewhere (Lehrach *et al.*, 1977).

Cloning and sequencing procedures

Poly(A)$^+$ mRNA was prepared using oligo(dT) columns and a cDNA library was constructed from this poly(A)$^+$ mRNA, using a cDNA synthesis kit (Pharmacia). The cDNA generated was ligated to λgt10 RI phage arms and packaged using an *in vitro* packaging kit (Amersham). Cloning procedures of recombinant phages were those previously described (Davis *et al.*, 1986). Double-stranded DNA was ligated to M13mp18 phage DNA digested with appropriate restriction enzymes and subsequently used for transformaion of *Escherichia coli* (Hanahan, 1985). Human placenta HpWE15 cosmid library (Clontech) was screened with the appropriate probes. Isolated clones were sequenced by the dideoxy chain termination procedure (Sanger *et al.*, 1977). Sequences were compared with genetic sequence data banks, GenBank (release 68) and EMBL (release 27) using GeneWorks software (IntelliGenetics, Inc.).

Probes

The IL2 cDNA probe, used throughout this study, was a 0.8 kb *Bam*HI fragment isolated from pAT153HIL2 plasmid, kindly provided by Dr Walter Fiers, State University of Ghent, Belgium. All the other probes used in this work are described in the text.

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Note added in proof

The sequence data reported here have been deposited in the EMBL sequence data library under the accession numbers Z14317 (Figure 3, line E), Z14318 (Figure 3, line D), Z14319 (Figure 3, line C), Z14320 (Figure 3, line A), Z14954 (BCM cDNA) and Z14955 (hybrid IL2–BCM cDNA).