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Structure and pre-B lymphocyte restricted expression of the *VpreB* gene in humans and conservation of its structure in other mammalian species

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DNA from several mammals, including humans, was found to contain one or more restriction enzyme digested DNA fragments which hybridized to the mouse *VpreB* gene under stringencies demonstrating at least 70% nucleotide sequence homologies, indicating that the *VpreB* locus may be widespread and highly conserved among mammals. A human *VpreB* genomic clone was isolated and sequenced. Two exons and the intervening intron are spaced almost identically as in the mouse *VpreB1* gene, and show 76% sequence homology to the mouse gene. As in the mouse *VpreB1* gene, the 5′ end of the human *VpreB* gene contains characteristic features of Ig domains, while the 3′ end is Ig non-related. This 3′ Ig non-related structure of the *VpreB* gene(s) may, therefore, have existed before the speciation of humans and mice over 65 million years ago. Sequences encoding the entire putative second framework region and a stretch in the third framework region are identical in human and mouse *VpreB* . The human *VpreB* gene appears to be selectively expressed in human pre-B cell lines as an 0.85 kb poly(A)$^+$ RNA. Its expression promises to be a useful marker for the detection of normal and malignant human pre-B lymphocytes.

Key words: B lineage restricted gene expression/human pre-B cell/Ig gene super family

Introduction

The expression of mRNA from two mouse genes, *VpreB1* and λ5, and possibly a third, *VpreB2*, has been associated primarily with pre-B cells (Sakaguchi *et al.*, 1986; Sakaguchi and Melchers, 1986; Kudo and Melchers, 1987). The λ5 gene 1.2 kb mRNA and the *VpreB* 850 bp mRNAs are useful pre-B cell differentiation stage-specific nucleic acid markers. The expression of λ5 and *VpreB1* (and/or *VpreB2*) may be related to regulatory events occurring in the ordered process of B cell differentiation and may be useful tools in further molecular dissection of B cell development. In both mouse and human, elucidation of the stages of B cell development has been based on studies of immunoglobulin gene rearrangement, the expression of cell surface and cytoplasmic markers, and by reactivities of cells to mitogenic or antigenic stimuli and growth factors (Tonegawa, 1983; Melchers *et al.*, 1977; Korsmeyer *et al.*, 1983; Nadler *et al.*, 1984; Foon and Todd, 1986). Transformed cell lines and tumors (mouse and human) and fresh leukemic samples (human) have been used as model systems to develop our understanding of B cell differentiation. We describe here the isolation and characterization of a human gene, *VpreB*, that is homologous to the mouse *VpreB1* gene and which so far appears to be selectively expressed in human pre-B cell lines. We also present preliminary evidence for a highly conserved homologue widely distributed through mammalian species which may allow identification, isolation and characterization of pre-B cells in many mammals.

Results

Detection of DNA sequences with homologies to the mouse VpreB genes in many mammalian species

A Southern blot survey of DNA from several species was conducted to determine if sequences homologous to the mouse *VpreB* genes (Kudo and Melchers, 1987) could be detected by cross-hybridization at various stringencies. Figure 1 shows the pattern of restriction fragments hybridiz-

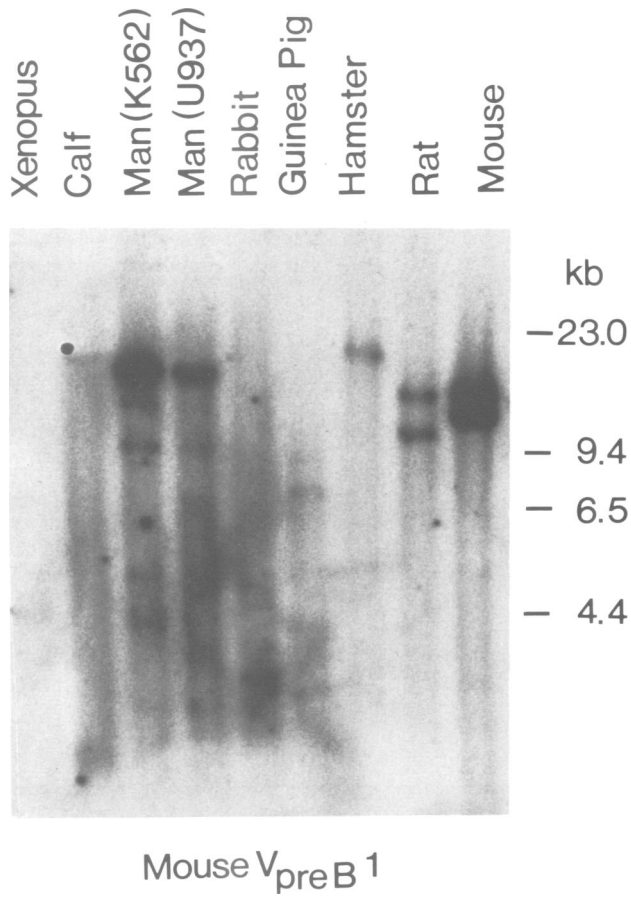

Fig. 1. Southern blot analysis of *Eco*RI-digested DNA from different species. Digested DNA (7 μg) from liver of rat, hamster, mouse, guinea pig and rabbit, from erythrocytes of frog, from calf thymus and from human cell lines was electrophoresed, blotted onto nitrocellulose, hybridized with a ^{32}P-labelled mouse *VpreB1* probe and washed under low stringency conditions as described in Materials and methods.

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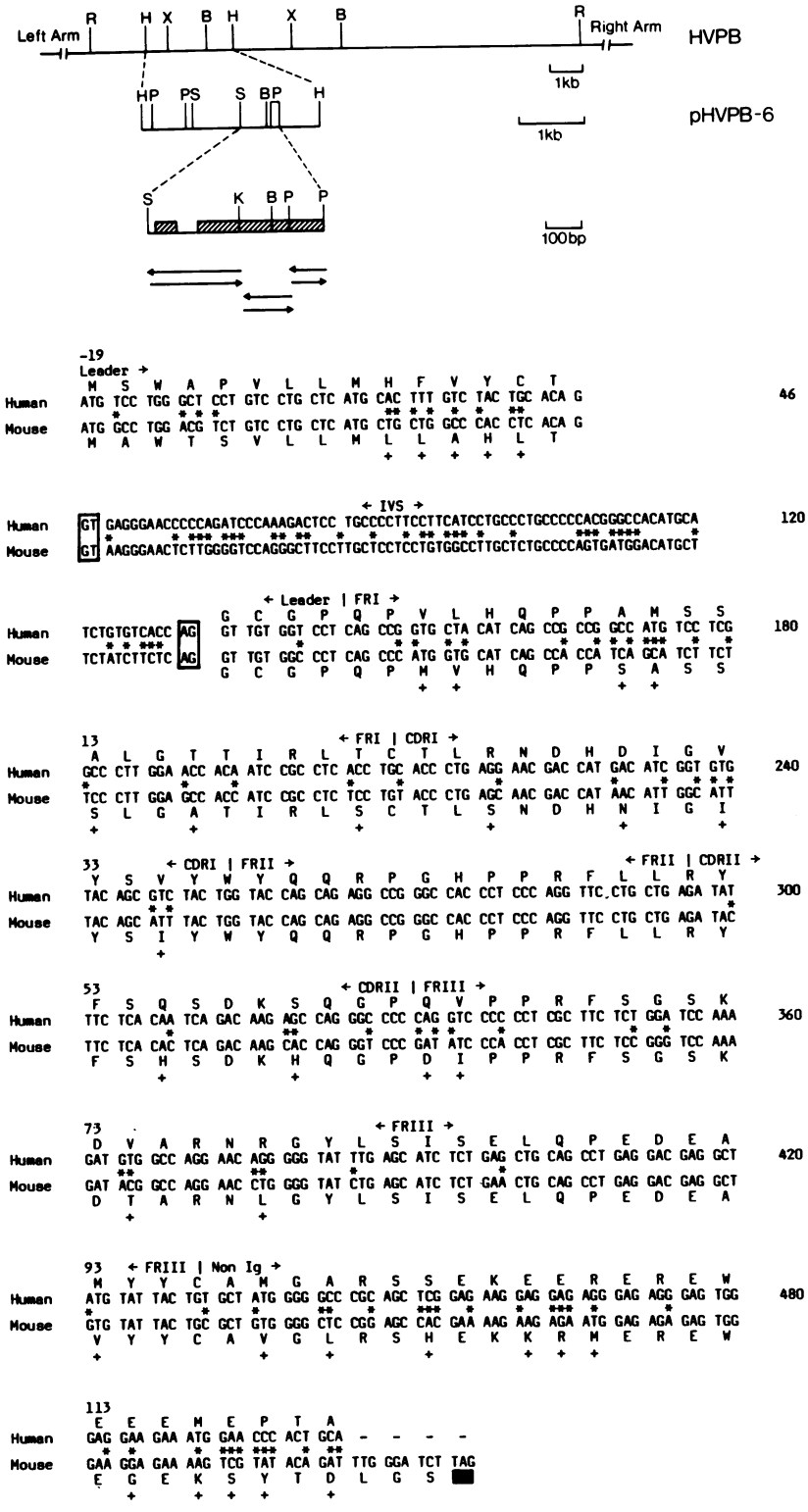

Fig. 2. Restriction map and sequencing strategy for HVPB phage clone and plasmid subclone pHVPB-6 and nucleotide sequence comparison of human *VpreB* and mouse *VpreB1* genes with their deduced amino acid sequences. Restriction enzyme sites are symbolized as: R = *Eco*RI; B = *Bam*HI; P = *Pst*I; K = *Kpn*I; S = *Sac*I; X = *Xba*I. Exons I and II are shown as rectangles enclosing slanted lines with the 5′ end at the left. Arrows show the direction and length of DNA sequences derived from indicated restriction fragments subcloned into M13. Putative exon structures are based on comparison with the sequence of mouse *VpreB1* cDNA (Kudo and Melchers, 1987). Potential splice sites are enclosed in boxes. An asterisk (*) indicates nucleotide differences while a plus (+) below the mouse amino acid residues indicates a putative protein difference. A black rectangle (■) indicates the mouse termination codon. Dashes (−) indicate human residues not yet determined. Numbering of deduced amino acid residues starts with −19 as the first position of the leader and proceeds from +1 as the first position of the putative mature protein (top left of each row). Nucleotide residues (right of each sequence row) begin with the A of the initiation codon. Note the 1 base gap in the IVS of the human sequence. Leader, CDR I and II, and FR I, II and III, indicate the locations of the leader, complementarity determining region, and framework regions typically found in immunoglobulin light chain variable regions (Kabat *et al.*, 1987) and following the alignment with V region genes of Kudo and Melchers, 1987.

ing at a stringency calculated to allow 30% mismatched base pairs (Wetmer and Davidson, 1968; Bonner *et al.*, 1973). Under these conditions DNA from all mammalian species of this survey hybridized to the mouse *VpreB1* probe indicating a widespread conservation of this sequence at a surprisingly high level of homology. At least one hybridizing band was detected in every species. In mouse two *VpreB* sequences with greater than 95% homology have been characterized (Kudo and Melchers, 1987). These sequences are on separate *Eco*RI fragments, giving rise to the two bands seen with mouse DNA in Figure 1. These results suggest that sequences with homologies to the mouse *VpreB* genes may be conserved in many species.

Table I. *VpreB* gene comparisons: percent sequence homology

	Leader	Intervening sequence	V-like	Non-Ig	Overall
Nucleotide	77	60	87	64	76
Amino acid	74	–	81	54	76

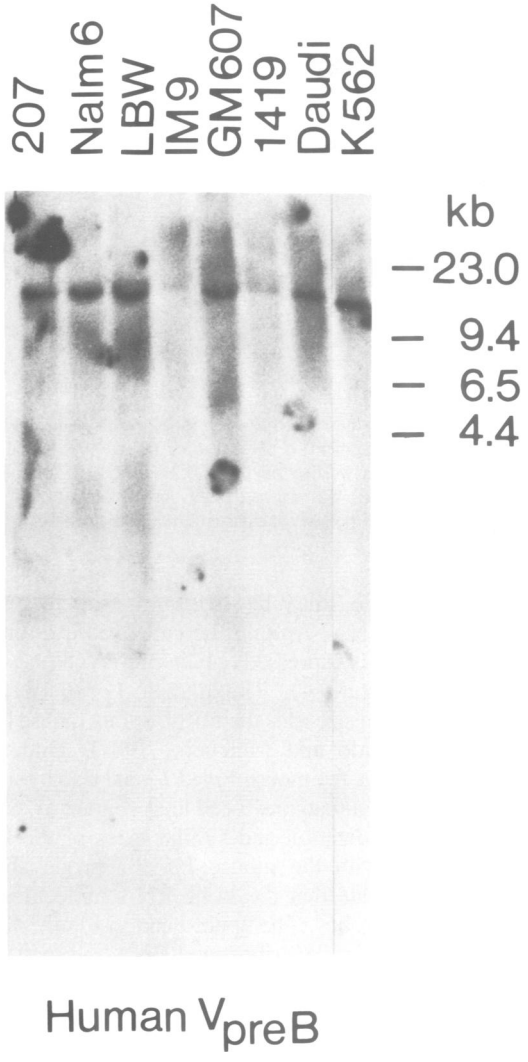

Fig. 3. Southern blot analysis of *Eco*RI-digested DNA from human cell lines. Digested DNA (7 μg) was applied to a 0.8% agarose gel, electrophoresed, then transferred onto nitrocellulose filter. The filter was hybridized with a ^{32}P-labelled 1.2 kb *Pst*I fragment of pHVPB-6 and washed under stringent conditions as described in Materials and methods. Sizes of hybridizing bands were calculated using *Hin*dIII-digested phage λ DNA as standards.

Isolation of human genomic DNA clones with sequences homologous to mouse VpreB

Human DNA contained a 15 kb *Eco*RI fragment that hybridized strongly to the mouse *VpreB1* probe (Figure 1). To determine the structure of this hybridizing fragment and its similarity with the mouse *VpreB* genes, a phage clone containing this 15 kb *Eco*RI fragment was isolated from a genomic library constructed from the human myeloid line U937. The library was screened with the mouse *VpreB1* probe used in the species Southern blot survey under the same stringency conditions (see Figure 1). Twenty positive clones were picked up in a screen of 3×10^6 clones. One of these clones, termed HVPB, was further characterized by plasmid subcloning, restriction mapping and DNA sequencing.

Structure and sequence of HVPB and comparison with other genes

Figure 2 shows the restriction map determined for the genomic phage clone HVPB and the plasmid subclone pHVPB-6. A 2.7 kb *Hin*dIII fragment that hybridized strongly to mouse *VpreB1* was subcloned in the *Hin*dIII site of pUC18. After more detailed restriction enzyme mapping, portions of this plasmid were further subcloned into M13 phage and sequenced according to the strategy shown in Figure 2. Altogether 0.5 kb of DNA spanning the regions of strongest homology between human *VpreB* and mouse *VpreB1* were sequenced. Alignment of the human and mouse nucleotide sequences revealed an overall homology of 76% (Figure 2). Striking features of the comparison include the identical length of the putative leader sequences which are encoded by exon I and the 5′ 11 bp of exon II, and the similar lengths of intron I and exon II. The exon–intron boundaries contain characteristic splice sequences (Brethnach and Chambon, 1981). A split codon 16, with the first nucleotide separated from the second and third by an intervening sequence, is present in human *VpreB* and is a typical feature of Ig gene family V-region leader sequences (Kabat *et al.*, 1987). Particularly noteworthy is that both the mouse and the human sequence diverge from typical V-region sequences at the same position, i.e. at the boundary to the CDR3 region of a typical V-gene. Comparison of these divergent sequences again show high homologies between mouse and human (Figure 2). The most remarkable feature in the similarities between mouse and human *VpreB* are the two stretches of identical nucleotide sequences spanning the whole putative framework II (nucleotide positions 250–299) and a 3′ region of the putative framework III (nucleotide positions 399–420). This identity in nucleotide sequences is matched by an identity of the putative amino acid sequences (Figure 2).

A computer search of a sequence databank (GenBank, Genetic Sequence Data Bank, Release 1986) revealed several regions within human *VpreB* homologous to portions of immunoglobulin variable region sequences. Between the putative amino acid residues 1 and 96, which constitute the mature V-region-like sequences, stretches of human and mouse V_H, V_κ and V_λ sequences showed between 35–50% amino acid homology to human *VpreB*. In contrast, mouse *VpreB1* and human *VpreB* are 81% homologous in this

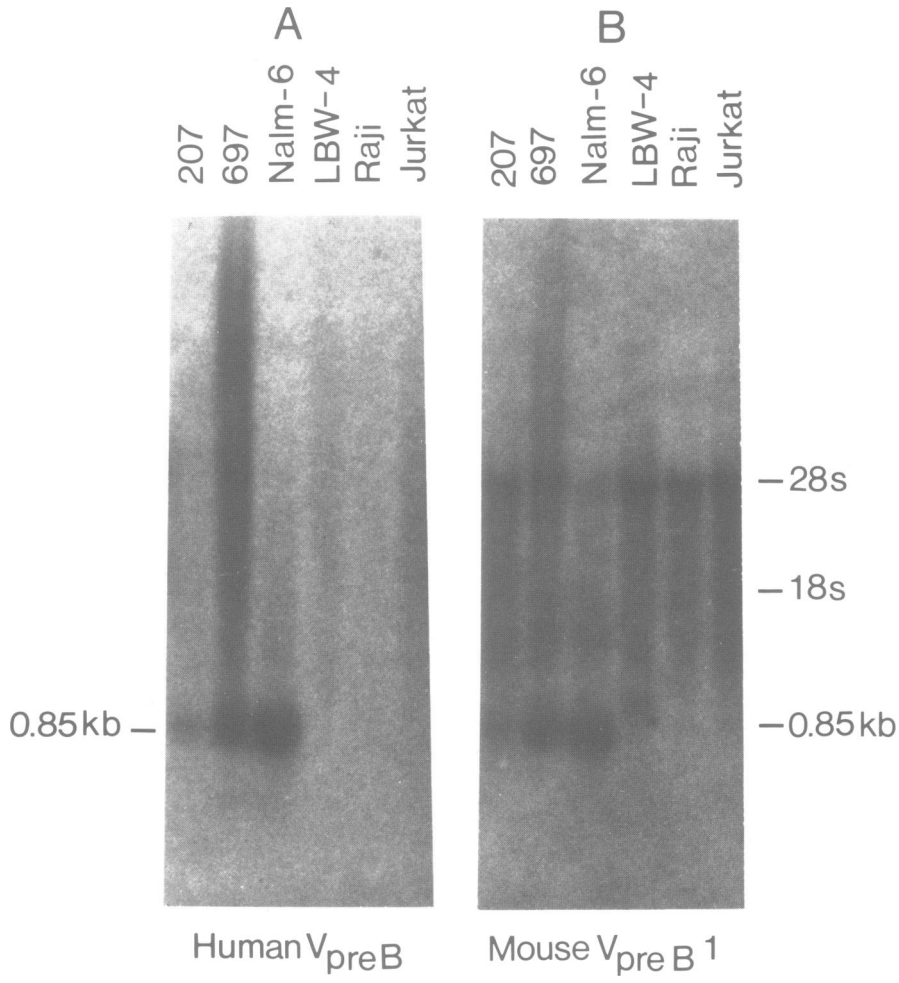

Fig. 4. Northern blot analysis of poly(A)-selected RNA from lymphoid cells. Poly(A)$^+$ RNA (5 μg) was applied to each lane, electrophoresed and blotted onto activated DPT paper. Identical filters were probed with: (**A**) a ^{32}P-labelled 1.2 kb *Pst*I fragment of pHVPB-6 or (**B**) a ^{32}P-labelled 560 bp *Eco*RI–*Acc*I fragment from pZ121, a mouse *VpreB1* cDNA clone. The filter in panel (**A**) was washed finally in 0.2× SSC, 0.1% SDS at 65°C, then exposed to X-ray film overnight at −80°C with intensifying screens. The filter in panel (**B**) was washed finally in 0.2× SSC, 0.1% SDS at 37°C and exposed as described above. Sizes of hybridizing bands were calculated using RNA mol. wt standards purchased from BRL (Bethesda, MD).

region (Table I). Nucleotide residues 400–430 showed 94% homology to V_λ gene 4A, subgroup VII (Anderson *et al.*, 1984), 87% homology to three V_H subgroup II genes (V_H32, V_H52 and H11; Matthyssens and Rabbitts, 1980; Rechavi *et al.*, 1982) and 79% homology to the V_H gene HIGI (Kudo *et al.*, 1985).

Genomic configuration of human VpreB gene in lymphoid cell lines

Figure 3 shows that no rearrangement of the human *VpreB* gene was detected in pre-B cells or in cells from later stages of B cell differentiation (LBW 4, GM109, Raji, Daudi) or in cells from myeloid (U937), erythroid (K562) or T (Jurkat) lineages. All of these samples had only the germline 15 kb *Eco*RI hybridizing fragment. Therefore, neither mouse nor human *VpreB* genes appear to be rearranged during B cell development.

Expression of human VpreB in lymphoid cell lines

One of the important characteristics of the mouse *VpreB1* gene is its restricted expression in mouse pre-B cell lines (Kudo *et al.*, 1987; Kudo and Melchers, 1987). We therefore examined the pattern of expression of human *VpreB* in human lymphoid lines by Northern blot analysis of poly(A)-selected RNA. Among the cell lines examined to date, human *VpreB* is expressed only in pre-B cell lines (207, 697, Nalm-6) (Figure 4). The human *VpreB* poly(A)$^+$ mRNA is 0.85 kb in size as is the mRNA of its mouse homologue *VpreB1* (Kudo and Melchers, 1987). Under low stringency conditions the mouse *VpreB1* gene also hybridizes to 0.85 kb RNA of human pre-B cell lines (Figure 4). Similar intensities of hybridization and similar sizes of the RNAs which hybridize with the mouse *VpreB1* probe and the human probe indicate that the same RNA molecules may hybridize to both probes. The upper band in Figure 4B corresponds to the size of 28S ribosomal RNA and may be the result of cross-hybridization of the mouse *VpreB1* probe to human ribosomal RNA at low stringency. The pattern of RNA expression of human *VpreB*, so far, follows that of *VpreB1* and λ5 in the mouse (Sakaguchi and Melchers, 1986) and indicates that human *VpreB* is selectively expressed in human pre-B cell lines but not in mature B cell or T cell lines.

Discussion

We have examined DNA from several species at different

evolutionary distances from mouse to see how widespread the pre-B lymphocyte-related *VpreB* locus (Kudo and Melchers, 1987) may have become during evolution, and how conserved its structure may have remained. Using a series of stringency conditions for DNA−DNA hybridization, we were surprised to find that at stringencies estimated to allow 30% base pair mismatching, the mouse *VpreB1* probe hybridized with at least one DNA fragment in all mammalian samples tested (Figure 1). Often two bands were observed perhaps indicating the existence of two *VpreB* genes in each of these species, similar to the situation in mouse which is known to carry two *VpreB* genes, *VpreB1* and *VpreB2* (Kudo and Melchers, 1987).

We think that the DNA fragments from the different species hybridizing with the mouse *VpreB1* probe are all close relatives to the *VpreB1* gene, much closer than the V gene segments which encode parts of the H- and L-chain variable regions. We believe this firstly because, the nucleotide sequences of the two mouse *VpreB* genes have much lower homologies to V-sequence genes of the H and L gene loci in mouse (i.e. between 46% and 57%, Kudo and Melchers, 1987) than the hybridization conditions of mouse *VpreB1* to DNA of other species indicate (i.e. around 70% homology for positive cross-hybridization). The presence of hybridizing DNA fragments at this stringency is a good indication of a high degree of evolutionary conservation.

Secondly we assume that the DNA sequences which cross-hybridized in the different species with the mouse *VpreB1* probe belong to a new locus of the Ig supergene family because of the structural similarity of the cross-hybridizing DNA sequences in one species, human, and compared with the mouse sequences. The structures of the human and mouse genes display several features typical of Ig V genes (Figure 2). These include a 19 amino acid putative leader sequence split by an intervening sequence of 87 bp that bridges a glycine codon interrupted after the first codon residue, and a glutamic acid putative amino terminus that often forms the capped residue found in V_λ genes (Kabat *et al.*, 1987). Immunoglobulin-like sequences are found up to the conserved cysteine that specifies the V region-like domain (Amzel and Poljak, 1979). Past this point, however, there is no homology with Ig genes, and this is so for mouse as well as human *VpreB* (Figure 2). The non Ig-like sequences at the 3′ end are again homologous between mouse and human. This is taken as strong evidence that the unusual 3′ end of *VpreB* has been together with the V-like 5′ end before the speciation of mouse and human over 65 million years ago (Klein, 1986).

Sequence comparison of human *VpreB* with mouse *VpreB1* detects surprisingly high sequence homologies in framework II and two parts of framework III not only at the amino acid, but also at the nucleotide sequence levels (Figure 2). There is total identity between bp 250−299, as well as between bp 399−420. The absence of the expected variation in third nucleotide positions of codons characteristic of silent mutations in areas of conserved protein structure with the same function in different species suggests that these regions of *VpreB* are conserved for selection on the DNA level. It may well be that these DNA regions bind regulatory proteins, a possibility which is being experimentally tested at present.

The mouse *VpreB1* gene is located only 4.6 kb away from another pre-B cell associated gene, λ5 (Kudo and Melchers, 1987), that resembles the mouse Cλ locus. The human homologue of λ5 has not yet been identified, because the hybridization across species with the λ5 probe appears not sufficiently high to detect the λ5 homologue in other species. A search for the human λ5 homologue at the appropriate 3′ location relative to the human *VpreB* is now under way.

It remains remarkable how varied the evolution of different parts of the *VpreB* gene appears that are contained in less than 2 kb of DNA. Its leader exon and the intron 3′ both show very strong homologies to λL chain leader sequences and their corresponding 3′ introns both in mouse (Kudo and Melchers, 1987) and in human (Figure 2, Table I). The V-segment-like 5′ part of the second exon of *VpreB*, however, again shows equal, and comparably lower, homologies to V_λ, $V_\varkappa$ and V_H gene segments in mouse as well as in humans. The 3′ end of the second exon of human *VpreB* shows homology to the 3′ end of the mouse *VpreB1* gene to an extent slightly lower than the homologies found between mouse and human leader sequences of the same gene.

Sequences of the putative framework II and of most of framework III of *VpreB*, on the other hand, are identical between mouse and human. All this indicates to us that very different evolutionary pressures have been exerted on these small regions in and around the *VpreB* gene. It therefore appears not possible to deduce an ancestral relationship of the *VpreB*/λ5 locus in relation to the λL chain locus, which has been estimated to develop from an ancestral gene ($c\lambda_0$) by duplications 240 million years ago (Selsing *et al.*, 1982).

The expression of human *VpreB* mRNA appears to be restricted to transformed human pre-B cell lines (Figure 4) although the sample is thus far limited in number. Previous studies on human B cell differentiation have focused on cell surface antigen expression and immunoglobulin gene rearrangement and expression in fresh leukaemic samples and in established B lineage cell lines (Nadler *et al.*, 1984; Korsmeyer *et al.*, 1983; Foon and Todd, 1986). Studies on the expression of human *VpreB* in a similarly large variety of cells and tissues is currently under way. The human *VpreB* gene, and eventually antibodies to its product, promise to become specific analytical tools to identify normal and malignant human pre-B lymphocytes.

Materials and methods

Cell lines and culture conditions

All human cell lines were maintained in RPMI 1640 containing 2 mM glutamine, 10% heat inactivated fetal calf serum (Gibco), 5×10^{-5} M β-mercaptoethanol, 100 μg/ml streptomycin and 200 U/ml penicillin at 37°C in a 10% CO_2 atmosphere. Pre-B cell lines 207, 697 (Findley *et al.*, 1982), Nalm-6 (Hurwitz *et al.*, 1979) and B cell line LBW-4 (Hendershott and Levitt, 1982) were gifts of Dr M.Cooper (University of Alabana, Birmingham, AL, USA). Multiple myeloma cell line IM-9 (De Meyts, 1976), B lymphoblastoid cell line 1419 and mature B cell line GM607 (Klobeck *et al.*, 1984) were gifts of Dr K.Willard-Gallo (International Institute of Cellular and Molecular Pathology, Brussels). Burkitts lymphoma cell lines Raji (Epstein and Barr, 1965) and Daudi (Klein *et al.*, 1968), myeloid line U937 (Sundström and Nilsson, 1976), erythroid line K562 (Andersson *et al.*, 1979) and T cell line Jurkat (Schneider *et al.*, 1977) were obtained from Dr N.Sakaguchi at our Institute.

Southern blot analysis

High mol. wt DNAs were extracted from human cell lines and from liver of mouse (C57BL/6), rat (Lewis), guinea pig (TRIK, Kleintierfarm Madörin AG, Füllinsdorf, Switzerland), rabbit (New Zealand white), hamster (Syrian) and from red blood cells of frog (*Xenopus laevis*). High mol. wt calf thymus DNA was bought from Pharmacia (Uppsala, Sweden). After restriction

enzyme digestion, 7 μg of DNA fragments were separated by electrophoresis on 0.7% agarose gels in TAE buffer (40 mM Tris−acetate, 1 mM EDTA) and transferred to nitrocellulose filters (BA85, Schleicher and Schüell) using 20× SSC (1× SSC = 150 mM NaCl, 15 mM sodium citrate) as transfer buffer (Southern, 1975). After transfer, filters were baked at 80°C under vacuum. Prehybridiztion and hybridization were done at 65°C in solutions containing 3× SSC, 10× Denhardt's solution (1× Denhardt's = 0.02% bovine serum albumin, 0.02% polyvinyl pyrolidone, 0.02% Ficoll), 0.1% SDS, 100 μg/ml salmon sperm DNA, and 1 mM EDTA. ^{32}P-labelled (Feinberg and Vogelstein, 1984) probes were used at 3 × 10^6 c.p.m./ml (Cerenkov counts). The 5′ *Eco*RI−*Acc*I 560 bp fragment of *VpreB1* contains all but the last 16 bases of putative amino acid coding region and was isolated from *VpreB1* cDNA plasmid clone pZ121 (Kudo and Melchers, 1987). The human *VpreB* probe was a 1.2 kb *Pst*I fragment isolated from pHVPB-6, a 2.7 kb *Hin*dIII pUC18 subclone of phage clone HVPB (Figure 2). Washing of the filters was done first at room temperature in 2× SSC, 0.1% SDS. For cross species hybridizations final washes were done with three 20 min washes in 1× SSC, 0.1% SDS at 65°C. Stringent washes were done in 0.2× SSC, 0.1% SDS at 65°C.

Northern blot analysis

Total RNA was isolated from cytoplasm after lysis of cells in 5% citric acid containing 0.1% NP-40 as described (Schibler *et al.*, 1980) and further purified by oligo(dT) cellulose chromatography as described (Sakaguchi *et al.*, 1986). Five micrograms poly(A)-enriched RNA was electrophoresed through 1% agarose gels containing 18 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 6% formaldehyde. Separated RNA was then blotted onto diazotized phenylthioether paper (Schleicher and Schüell). Prehybridization of filters was done at 45°C in solutions containing 5× SSPE (1× SSPE = 150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA), 5× Denhardt's, 2% glycine, 50% deionized formamide, 100 μg/ml salmon sperm DNA, 20 μg/ml yeast tRNA and 1 μg/ml poly(A). Stringent hybridizations were done at 45°C in prehybridization solution lacking glycine but containing 10% Dextran sulfate and 3 × 10^6 c.p.m./ml ^{32}P-labelled probe. Cross species hybridizations were done at 37°C in hybridization solution containing only 30% formamide. Stringent washes were done at 65°C in 0.2× SSC, 0.1% SDS. Cross species hybridization experiments were washed finally in 0.2× SSC, 0.1% SDS at 37°C.

Isolation and characterization of human VpreB genomic clones

DNA from human myeloid line U937 was digested to completion with *Eco*RI. Ten micrograms of digested DNA was separated by electrophoresis through 1% low melting point agarose (Bethesda Research Laboratories). DNA fragments ranging from 10−16 kb were excised from the gel and purified by organic solvent extractions and ethanol precipitation. Purified, size-fractionated U937 DNA was used to construct a genomic phage library in the vector λgt λwes B (Leder *et al.*, 1977). The partial human genomic library was screened with ^{32}P-labelled *Eco*RI−*Acc*I fragment of mouse *VpreB1* cDNA pZ121 under conditions of hybridization and washing used for cross species hybridization of Southern blots described above. One positive clone, HVPB, was used to isolate a 2.7 kb *Hin*dIII fragment that hybridized strongly to the mouse *VpreB1* cDNA probe. This fragment was inserted into the *Hin*dIII site of plasmid pUC18 to derive recombinant plasmid pHVPB-6.

DNA sequencing was performed on M13 mp18 and mp19 subclones of pHVPB-6 by the dideoxy chain termination method (Sanger *et al.*, 1977) using a universal M13 17-mer primer.

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References

Amzel,L.M. and Poljak,R. (1979) *Annu. Rev. Biochem.*, **48**, 961−997.
Anderson,M.L.M., Szajnert,M.F., Kaplan,J.C., McColl,L. and Young,G.B. (1984) *Nucleic Acids Res.*, **12**, 6647−6661.
Andersson,L.C., Nilsson,K. and Gahmberg,C.G. (1979) *Int. J. Cancer*, **23**, 143−147.
Bonner,T.I., Brenner,D.J., Neufeld,B.R. and Britten,R.J. (1973) *J. Mol. Biol.*, **81**, 123−135.
Breathnach,R. and Chambon,P. (1981) *Annu. Rev. Biochem.*, **50**, 349−383.
De Meyts,P. (1976) In Blecker,M. (ed.), *Methods in Receptor Research, Part I.* Marcel Dekker, Inc., New York, pp. 309−310.
Epstein,M.A. and Barr,Y.M. (1965) *J. Nat. Cancer Inst.*, **34**, 231−240.
Feinberg,A.P. and Vogelstein,B. (1984) *Anal. Biochem.*, **137**, 266−267.
Findley,H.W., Cooper,M.D., Kim,T.H., Alvarado,C. and Ranjab,A. (1982) *Blood*, **60**, 1305−1309.
Foon,K.A. and Todd,R.F. (1986) *Blood*, **68**, 1−31.
Hendershot,L. and Levitt,D. (1982) *J. Exp. Med.*, **156**, 1622−1634.
Hurwitz,R., Hozier,J., LeBien,T., Minowada,J., Gajl-Peczalska,K., Kuboniski,I. and Kessey,J. (1979) *Int. J. Cancer*, **23**, 174−180.
Kabat,E.A., Wu,T.T., Bilofsky,H., Reid-Miller,M. and Perry,H. (1987) In *Sequences of Proteins of Immunological Interest.* US Department of Health and Human Services, Washington, D.C., USA.
Klein,J. (1986) In *Natural History of the Major Histocompatibility Complex.* John Wiley and Sons, New York, p. 718.
Klein,E., Klein,G., Nadkarni,J.S. *et al.* (1968) *Cancer Res.*, **28**, 1300−1310.
Klobeck,H.-G., Solomon,A. and Zachau,H.G. (1984) *Nature*, **309**, 73−76.
Korsmeyer,S.J., Arnold,A., Bakshi,A., Ravetch,J.V., Siebenlist,U., Heiter,P.A., Sharrow,S.O., LeBien,T., Kessey,J.H., Poplack,D.G., Leder,P. and Waldmann,T.A. (1983) *J. Clin. Invest.*, **71**, 301−313.
Kudo,A. and Melchers,F. (1987) *EMBO J.*, **6**, 2267−2272.
Kudo,A., Ishihara,T., Nishimura,Y. and Watanabe,T. (1985) *Gene*, **33**, 181−189.
Kudo,A., Sakaguchi,N. and Melchers,F. (1987) *EMBO J.*, **6**, 103−107.
Leder,P., Tiemeier,D. and Enquist,L. (1977) *Science*, **196**, 175−177.
Matthyssens,G. and Rabbitts,T. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 6561−6565.
Melchers,F., Andersson,J. and Phillips,R.A. (1977) *Cold Spring Harbor Symp. Quant. Biol.*, **41**, 147−158.
Nadler,L.M., Korsmeyer,S.J., Anderson,K.C., Boyd,A.W., Slanghenhaupt,B., Park,E., Jensen,J., Coral,F., Mayer,R.J., Sallan,S.E., Ritz,J. and Schlossman,S.F. (1984) *J. Clin. Invest.*, **74**, 332−340.
Rechavi,G., Bienz,B., Ram,D., Ben-Neziah,Y., Cohen,J.B., Zakut,R. and Givol,D. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4405−4409.
Sakaguchi,N., Berger,C.N. and Melchers,F. (1986) *EMBO J.*, **5**, 2139−2147.
Sakaguchi,N. and Melchers,F. (1986) *Nature*, **324**, 579−582.
Sanger,F., Nicklen,S. and Coulson,A. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463−5487.
Schibler,U., Tosi,U., Pittet,A., Fabiani,L. and Wellauer,P.K. (1980) *J. Mol. Biol.*, **142**, 93−116.
Schneider,U., Schwenk,H.U. and Bornkamm,G. (1977) *Int. J. Cancer*, **19**, 521−526.
Selsing,E., Miller,J., Miller,R. and Storb,U. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4681−4685.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503−517.
Sundström,C. and Nilsson,K. (1976) *Int. J. Cancer*, **17**, 565−577.
Tonegawa,S. (1983) *Nature*, **302**, 575−581.
Wetmer,R. and Davidson,N. (1968) *J. Mol. Biol.*, **31**, 349−370.

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