The EMBO Journal vol.13 no.24 pp.5937–5943, 1994

A surrogate 15 kDa JCκ protein is expressed in combination with μ heavy chain by human B cell precursors

Veronique Francés, Dominique Pandrau-Garcia, Christiane Guret, Stephen Ho, Zhixiong Wang, Valérie Duvert, Sem Saeland and Héctor Martinez-Valdez[1]

Schering-Plough, Laboratory for Immunology Research, Dardilly, France
[1]Corresponding author

Communicated by K.Rajewsky

A novel κ protein, encoded by a germline JCκ transcript, is expressed by normal and leukemic human B cell precursors. The transcript displays an open reading frame initiated by a non-AUG codon, and predicts a 15 kDa molecule which could be readily confirmed by *in vitro* translation. Cellular expression was demonstrated by immunofluorescence, precipitation and Western blotting. Furthermore, 2-D gel electrophoresis revealed that germline JCκ can covalently associate with μ heavy chain at the surface of pre-B cells. We therefore propose that during B cell lymphopoiesis, two alternative pathways could be operative in which μ heavy chain can either associate with λ5 or germline JCκ.

Key words: B lymphopoiesis/cDNA cloning/immunoglobulin rearrangement/germline κ

Introduction

During the development of B lymphocytes, gene segments on the immunoglobulin (Ig) heavy (H) and light (L) chain loci assemble in a defined ordered manner (Alt *et al.*, 1981; Tonegawa, 1983). Thus, germline B cell progenitors (pro-B cells) rearrange D_H and J_H genes (pre-B1 cells), and subsequently join a given V_H gene to the rearranged D_H-J_H segment (pre-B2 cells), allowing the expression of μ_H chain (Alt *et al.*, 1992; see a review in Cooper and Burrows, 1990). Finally, if a successful V_L to J_L rearrangement occurs, the cells can express κ or λ chains and can mature into B cells, displaying surface IgM antigen receptor. Studies focusing on the molecular mechanisms governing Ig rearrangement have suggested that the expression of μ_H at the surface of pre-B cells, in association with surrogate λ5 and Vpre-B (Kerr *et al.*, 1989; Karasuyama *et al.*, 1990; Bossy *et al.*, 1991) molecules (ψλL), controls the assembly of light chain genes (Reth *et al.*, 1987; Tsubata *et al.*, 1992). This complex represents a pre-B cell surface receptor, which is proposed to be essential for the transduction of signals that initiate L chain rearrangement (Tsubata *et al.*, 1992). Recently, however, gene targeting at the λ5 locus did not completely abrogate B cell development (Kitamura *et al.*, 1992; Rolink *et al.*, 1993), suggesting the possibility that another gene which functions similarly to λ5 may alternatively associate with μ_H. In this work we report the cloning of a germline JCκ cDNA encoding a 15 kDa protein, which has the capacity to associate with μ_H chain at the surface of B cell precursors.

Results

B cell precursors express unrearranged κ transcripts

Analogous to previous reports in the murine (Leclerq *et al.*, 1989) and human (Thompson *et al.*, 1992) systems, the B cell precursor acute lymphocytic leukemia (BCP-ALL) cell line pre-ALP, established recently in our laboratory (Pandrau *et al.*, 1993), expressed germline κ transcripts. cDNA cloning and sequencing revealed that the germline κ transcript displayed an unrearranged Jκ1 region accurately spliced to the Cκ region (data not shown). To corroborate further that the transcript originated from an unrearranged κ locus, we analyzed the configuration of both κ and λ genes. Following endonuclease digestion and Southern blotting, a structural comparison between pre-ALP and κ- (Daudi and the B207 variant) or

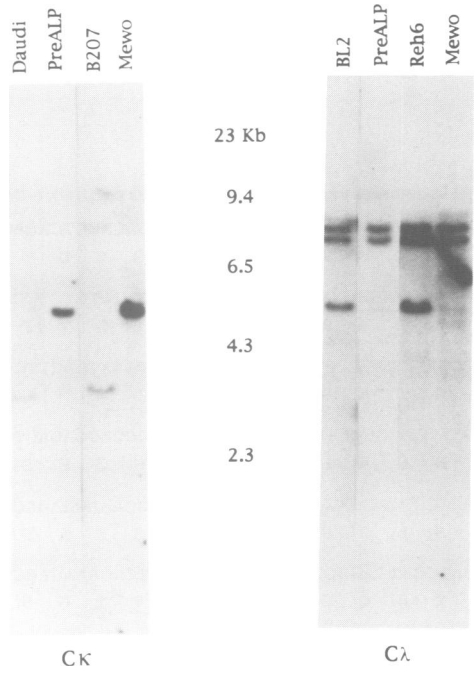

Fig. 1. Organization of the κ and λ gene loci in pre-ALP cells. A structural comparison is shown, by Southern blotting at the κ and λ loci, between cell lines that have undergone rearrangement (Daudi and B207 for the κ, and BL2 and Reh6 for the λ genes, respectively) and pre-ALP. The melanoma cell line Mewo was used as a control for germline configuration.

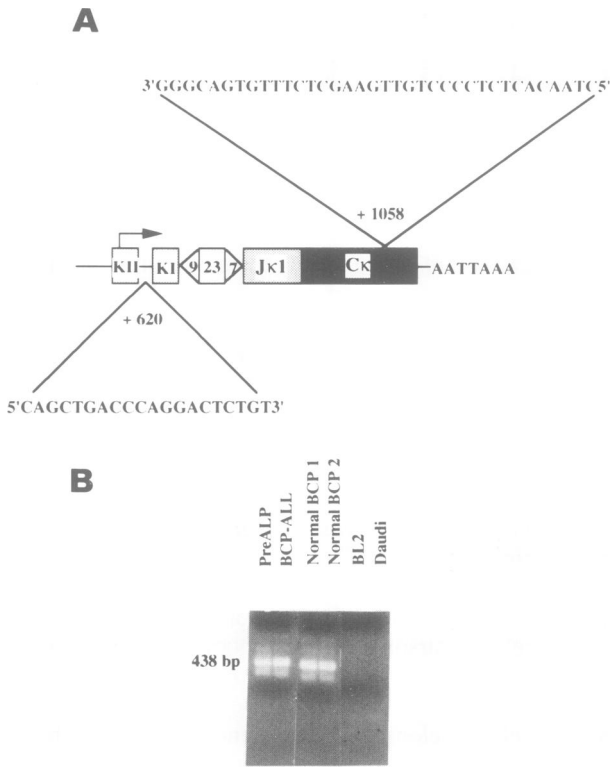

Fig. 2. Comparative RT-PCR analysis of Pre-ALP's germline JCκ transcript expression, with other BCP-ALL cells, normal B cell precursors and mature B cell lines. (**A**) Schematic structure of the mRNA template is shown, as well as the location and sequence of amplifying primers which predicts a 438 bp fragment. (**B**) The results following amplification. As indicated, both pre-B ALL and normal B cell precursor mRNA templates generated the same JCκ PCR product as pre-ALP. Size was confirmed by comparison with molecular markers, and the specificity as a B cell precursor transcript was corroborated by absence in the mature B cell lines, Daudi and BL2.

λ-expressing (BL2 and Reh6) mature B cell lines was carried out. As a control for germline configuration, the melanoma cell line Mewo was used. Figure 1 demonstrates that pre-ALP has both the κ and λ gene loci in germline configuration, and confirms the unrearranged nature of the JCκ transcript.

JCκ transcript can be detected in normal and leukemic B cell precursors

To examine whether the expression of JCκ occurs during normal B cell precursor development, a PCR strategy was devised based on the structural organization of the germline κ mRNA (Figure 2A). We thus designed amplifying primers, spanning a 438 bp segment between the K° region (Van Ness *et al.*, 1981) and Cκ domain. As depicted in Figure 2B, in addition to pre-ALP, other BCP-ALL cells as well as normal $CD10^+$ $sIgM^-$ B cell precursors expressed the JCκ transcripts, supporting the hypothesis that JCκ expression also occurs during normal B cell lymphopoiesis. Furthermore, the mature B lymphocyte cell lines Daudi and BL2, which have undergone rearrangement of either their κ or λ loci (Figure 1), respectively, did not express germline κ transcripts, indicating that the expression of JCκ is a feature of B cell precursors.

Germline JCκ cDNA sequence encodes a 15 kDa protein

As no classic AUG codon followed by an open reading frame (ORF) could be identified, it was concluded previously that the expression of germline JCκ is restricted to transcription (Leclerq *et al.*, 1989; Thompson *et al.*, 1992). Given the structural analogy with the JCλ homologue λ5, we hypothesized, however, that JCκ might be productively translated using an alternative translational initiation codon. In this context, *in vitro* studies have shown previ-

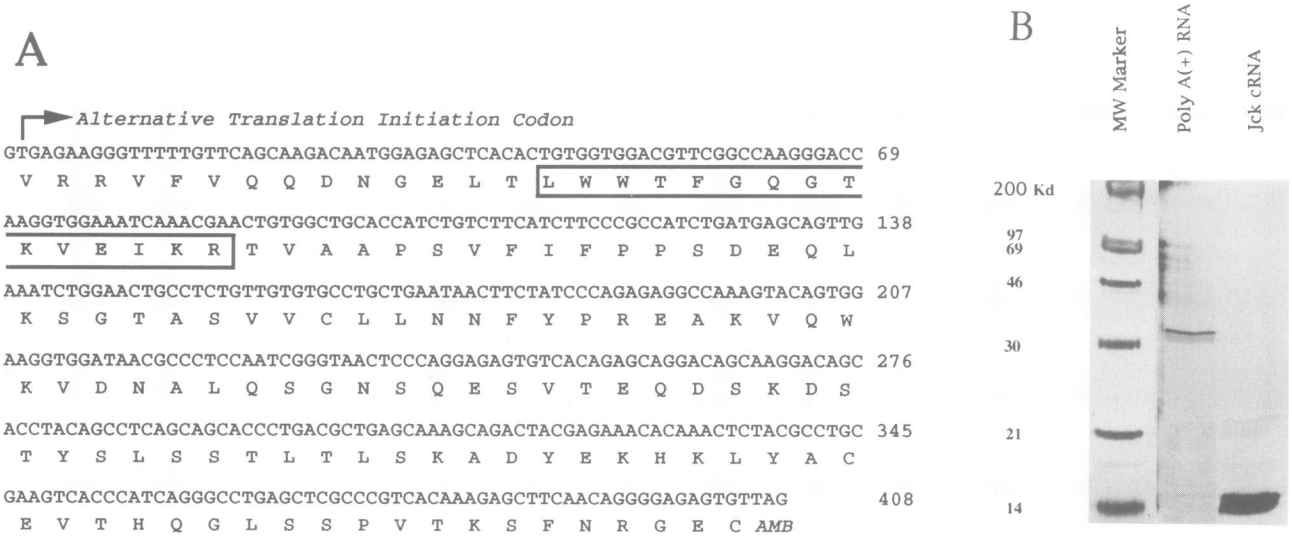

Fig. 3. Cloned cDNA encodes a germline JCκ protein. (**A**) Using the Cκ probe, a pre-ALP cDNA library was screened and clones corresponding to germline JCκ were identified. Sequence analysis revealed a non-rearranged Jκ1 region accurately spliced to Cκ. Shown here is the sequence corresponding to the putative ORF (a complete sequence has been reported previously; Thompson *et al.*, 1992). The boxed amino acid sequence depicts the peptide chosen to generate an anti-JCκ antibody. An alternative translation initiation codon GUG, shown here as GTG according to its cDNA designation (indicated by the arrow), is proposed to precede an ORF which deduces a germline JCκ protein of 15 kDa. (**B**) *In vitro* translation of the 15 kDa JCκ molecule. Autoradiogram of ^{35}S-labelled proteins following *in vitro* synthesis. The analysis was performed using cRNA derived from the JCκ cDNA template. As a control for the reaction efficiency, 1/10 of a translation assay using a poly(A)$^+$ RNA was analyzed.

ously that tRNA can potentially bind and initiate protein synthesis at non-canonic translational initiation codons (Hann *et al.*, 1988; Bernards and de la Monte, 1990). Thus, in addition to AUG, GUG (1/30 times as frequently as classic AUG), UUG and CUG can be used. In agreement with this notion, the JCκ cDNA sequence depicted in Figure 3A displays an alternative translation initiation GUG codon, revealing an ORF which predicted a germline κ protein of ~15 kDa. To confirm whether germline JCκ transcript encoded the 15 kDa molecule, *in vitro* translation experiments were carried out following the generation of cRNA from the JCκ cDNA template. Figure 3B shows that consistent with the deduced germline κ molecule, *in vitro* protein synthesis revealed a polypeptide of ~15 kDa which is in agreement with the size predicted from the cDNA reading frame. Taken together, these data strongly suggest that surrogate germline JCκ could be productively expressed by B cell precursors.

Intracytoplasmic expression of surrogate JCκ by pre-B ALL cell lines

We have shown that both pre-ALP cells, as well as other pre-B ALL cell lines, express the JCκ transcript, and have demonstrated by *in vitro* translation that it encodes a 15 kDa germline κ molecule. We therefore investigated whether this protein could be expressed at the cellular level. Thus, an antibody to a peptide spanning a segment between germline κ region and the Jκ1 domain (Figure 3A) was generated and its reactivity tested on B lineage cells by immunofluorescence and precipitation. As illustrated in Figure 4 a, pre-ALP cells and the cell line Blin-1, reported previously as a pre-B ALL-expressing JCκ transcript (Martin *et al.*, 1991), stained intracytoplasmically with the anti-JCκ antibody. In contrast, a mature variant of the B207 cell line (Findley *et al.*, 1982), which has undergone rearrangement of its κ locus (shown in Figure 1), did not react with the anti-peptide, demonstrating that the anti-JCκ antibody recognizes a B cell precursor product. For comparison, we tested a panel of commercially available anti-κ monoclonal antibodies (mAbs) and identified two with distinctive reactivities. One anti-κ mAb stained germline JCκ protein in both pre-ALP and Blin-1 cells, but in addition reacted with rearranged κ in B207 (Figure 4b). The second mAb, which exclusively recognized rearranged κ molecules, positively labelled B207 cells but failed to react with either pre-ALP or Blin-1 (Figure 4c).

Immunoprecipitation and Western blotting confirm the expression of the 15 kDa JCκ molecule by B cell precursors

To prove that the protein stained with the anti-JCκ antibody is indeed the 15 kDa molecule, comparative immunoprecipitation studies were carried out following ^{35}S metabolic labelling of pre-ALP and B207 cells. This was followed by SDS−PAGE. As shown in Figure 5A, the anti-JCκ antibody precipitated a 15 kDa molecule from pre-ALP but not from B207 extracts. To demonstrate further that intracytoplasmic staining of pre-B cells is exclusively due to reactivity to the germline κ molecule, we performed a Western blot experiment using the monoclonal anti-κ mAb which recognizes both germline and rearranged products. As shown in Figure 5B, following

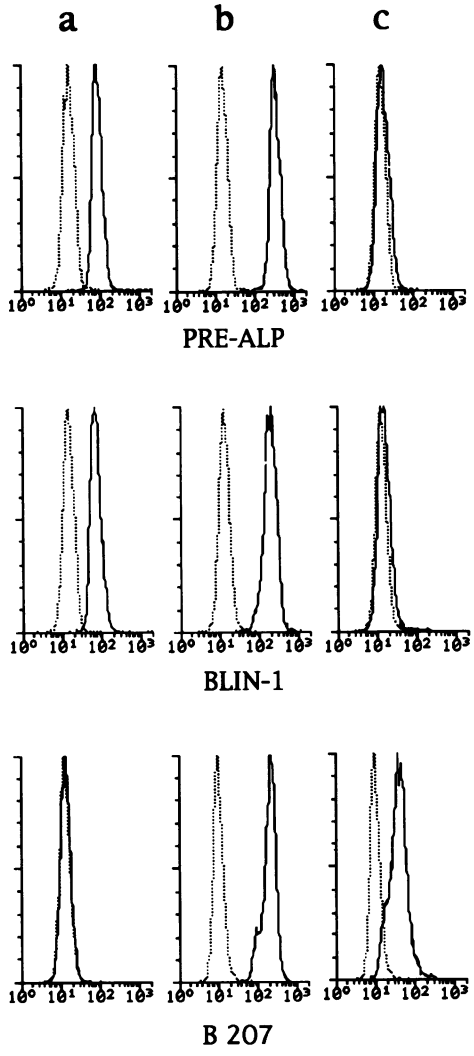

Fig. 4. Identification of germline JCκ protein in B cell precursors. Immunofluorescence analysis of the pre-B ALL cell lines pre-ALP and Blin-1 and a κ-rearranged variant of the B207 cell line. (**a**) Reactivity of the anti-JCκ-specific (protein-G purified) antibody generated by immunization of rabbits with a synthetic JCκ peptide (boxed in Figure 3A). (**b**) Staining with an anti-κ mAb (Silenus Laboratories) which reacts with both germline and rearranged products. (**c**) Analysis using an anti-κ mAb with exclusive specificity for rearranged κ (Ortho Diagnostic Systems).

SDS−PAGE and transfer of a protein extract from the cell line pre-ALP, a unique band of 15 kDa was obtained, matching the size of the product generated by *in vitro* translation and by specific anti-JCκ immunoprecipitation. Comparatively, as a control experiment, the reactivity of the anti-κ mAb with the rearranged κ light chain was corroborated by its binding to the 25 kDa κ molecule (Figure 5B).

Normal B cell precursors (BCP) express intracytoplasmic JCκ

We have shown previously that normal pre-B cells express germline JCκ transcripts (Figure 2B). As we have demonstrated above (Figures 4 and 5B) that the monoclonal anti-κ antibody recognizes both the 15 kDa germline JCκ and the rearranged 25 kDa molecules, we exploited the reactivity of this mAb to examine the expression of the JCκ molecule by normal B cell precursors. Figure 6A

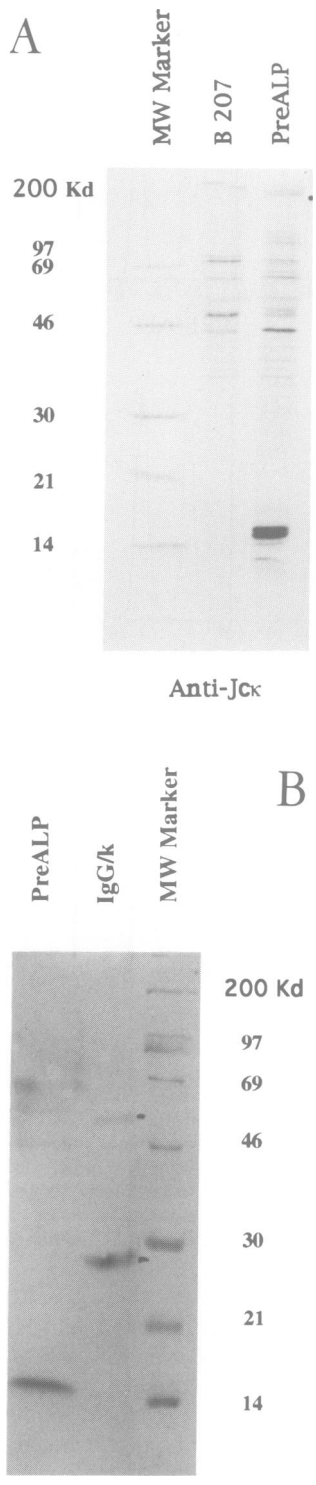

Fig. 5. JCκ encodes a 15 kDa molecule. (**A**) Immunoprecipitation of ^{35}S metabolically labelled pre-ALP and B207 cell lysates (see Materials and methods) using anti-JCκ-specific serum. (**B**) Western blot showing dual reactivity of the Silenus anti-κ mAb to both germline and rearranged moieties. Lane 1 corresponds to a pre-ALP lysate, while lane 2 is a conventional IgG-κ control molecule.

depicts the intracytoplasmic fluorescent staining which revealed that normal $CD10^+$ $sIgM^-$ BCPs express the JCκ protein. In contrast, and as shown in Figure 6B, the reactivity to the mAb which exclusively detects rearranged κ molecules (corroborated by the surface staining of the

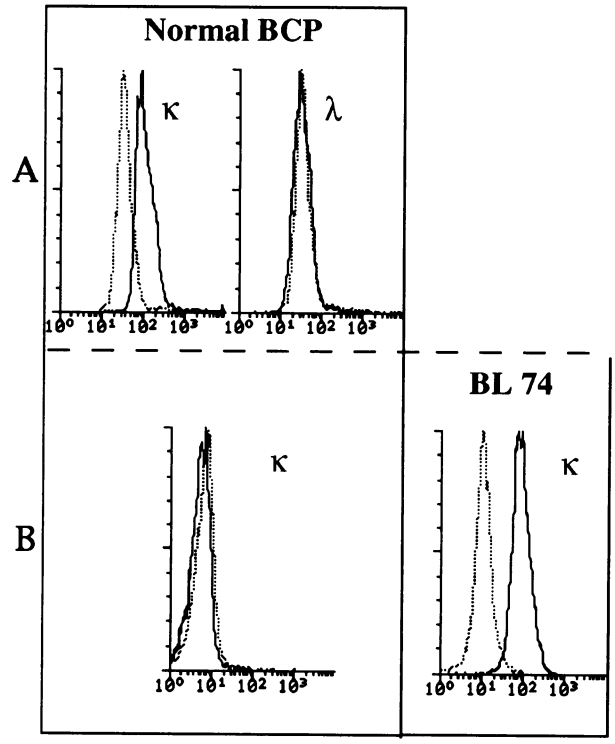

Fig. 6. Immunofluorescence κ reactivity of normal B cell precursors. (**A**) Fetal B cell precursors ($CD10^+$ sIg^-) were tested for intracytoplasmic reactivity with anti-κ mAb (Silenus Laboratories), which stains both germline and rearranged moieties, following membrane permeabilization. Potential contamination with mature B cells was internally controlled by staining with a conventional anti-λ mAb (Immunotech SA, Marseille, France). (**B**) Surface expression was monitored with the anti-κ mAb, which exclusively reacts with rearranged κ proteins (Ortho Diagnostic Systems), and using as a control the mature B cell line BL74 (a kind gift from Prof. G.Lenoir, CIRC, Lyon, France). Negative fluorescence control (dotted line) was obtained with a murine mAb of unrelated specificity.

mature B cell line BL74) was consistently negative. In addition, intracytoplasmic λ expression, tested with a conventional anti-λ mAb, was also negative. These data indicate that intracytoplasmic reactivity to the anti-κ mAb in normal B cell precursors is due to the expression of surrogate ψkLC molecule.

Germline JCκ has the capacity to covalently associate with μ heavy chain at the surface of B cell precursors

A number of studies indicate that the association of μ to surrogate λ5 and Vpre-B is an important event during B cell ontogeny (Misener *et al.*, 1991; Bossy *et al.*, 1993; Brouns *et al.*, 1993). Our present results suggest that such an association may not be restricted to λ5 gene products. To investigate whether surrogate JCκ can be associated with μ heavy chain, immunoprecipitation experiments were carried out following ^{35}S metabolic labelling of the pre-ALP cells and resolved by SDS–PAGE. As depicted in Figure 7A, when pre-ALP proteins were analyzed after reaction with an anti-μ antibody, co-precipitation of the 15 kDa JCκ together with μ_H could be readily determined. In contrast, the pre-B leukemia Nalm6 (Hurwitz *et al.*, 1979) expressed μ_H in association with 22 kDa λ5 and 18 kDa Vpre-B (as described previously by Kerr *et al.*,

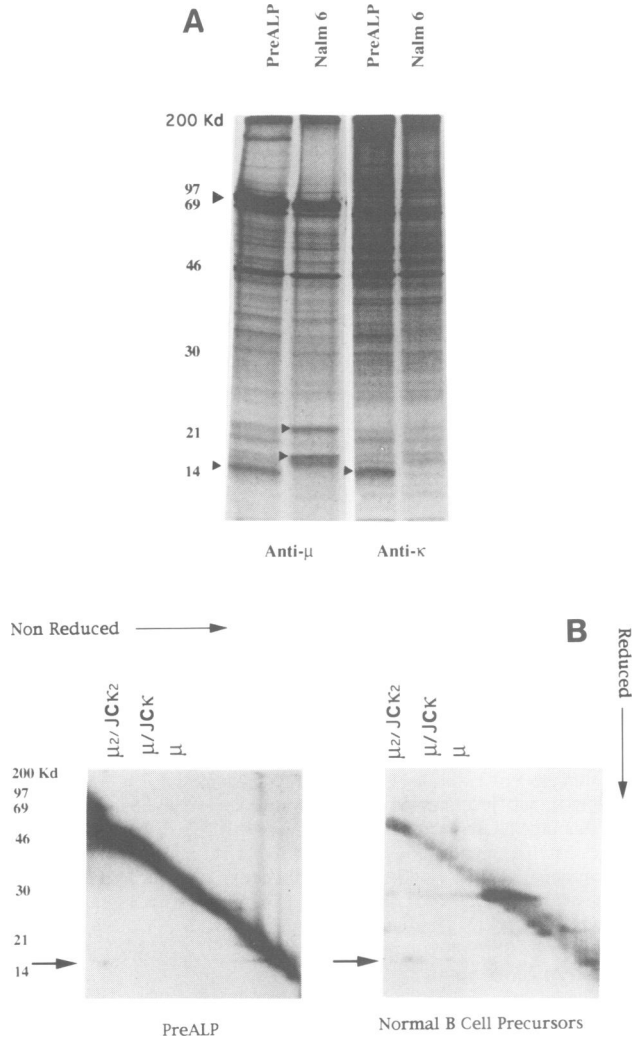

Fig. 7. Association of μ_H with surrogate light chain proteins. (**A**) Immunoprecipitation of pre-ALP and Nalm6 ^{35}S-labelled cell lysates using either anti-μ or anti-κ mAbs. μ_HC, ψκL (JCκ) and ψλL (λ5 and Vpre-B) are indicated by arrows. (**B**) 2-D (non-reducing/reducing) gel electrophoresis analysis following precipitation of surface-iodinated pre-ALP and normal B cell precursor cell lysates.

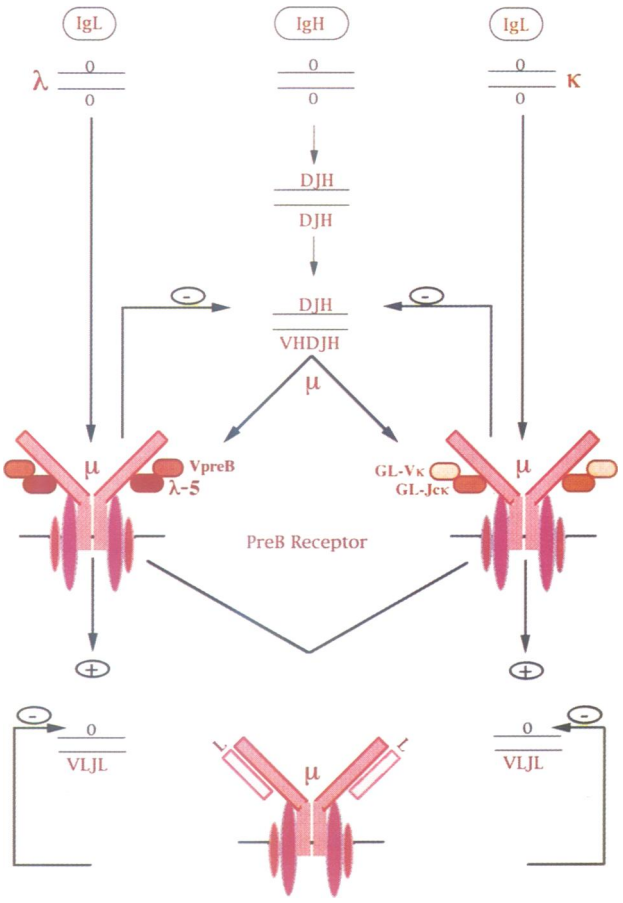

Fig. 8. Hypothetical model suggesting alternative pathways of B cell precursor differentiation. A stepwise progression of Ig gene rearrangement is presented. Thus, upon heavy chain rearrangement, μ_H can alternatively associate with λ5 and Vpre-B molecules, or with germline κ products (both germline λ and κ products are represented in red and orange, respectively). Such association, in combination with the α and β Ig chains (depicted in the diagram as membrane-bound, oval-shaped and in dark and light pink), proposes two alternative forms of pre-B cell receptors. The formation of the pre-B receptor is presumably essential for the transduction of signals (Takemori *et al.*, 1990) which results in heavy chain allelic exclusion and induction of light chain rearrangement.

1989), with no evidence of the 15 kDa JCκ molecule. Moreover, immunoprecipitation with an anti-κ mAb showed that the association of μ_H with germline JCκ could only be detected in pre-ALP and not in Nalm6. It is worth noting that major expression of surrogate JCκ is detected intracytoplasmically, presumably as a free molecule. This is reflected by the lower μ_HC moiety in the anti-κ precipitates. Therefore, to investigate directly whether a ψκL–μ_H complex could be determined at the cell surface, a 2-D (non-reduced/reduced) SDS–PAGE was carried out following anti-κ immunoprecipitation of surface iodinated proteins. Thus, Figure 7B shows that the 15 kDa germline JCκ protein dissociates from the μ heavy chain moiety, both in pre-ALP and normal BCP cells, after the reduction of disulfide bonds. Taken together, these data demonstrate that μ and JCκ proteins can covalently associate at the cell surface of human pre-B cells.

Discussion

Germline light chain λ5 and Vpre-B (ψLC) genes were discovered (Sakaguchi and Melchers, 1986; Kudo and Melchers, 1987) as a result of their selective transcription in murine B cell precursors, followed by the identification of the human 14.1 and Vpre-B counterparts (Chang *et al.*, 1986; Hollis *et al.*, 1989; Bossy *et al.*, 1991). As revealed from structural studies, these genes have significant homology with the conventional λLC, but differ in that they do not undergo rearrangement. While the λ5/14.1 are homologues of the Jλ–Cλ genes (Pillai and Baltimore, 1987; Hollis *et al.*, 1989) encoding a 22 kDa protein, the Vpre-B genes are highly homologous to Vλ and encode an 18 kDa molecule (Kudo and Melchers, 1987; Pillai and Baltimore, 1988). In this work we report the identification and characterization of a 15 kDa germline JCκ protein. This molecule is the product of unrearranged Jκ–Cκ genes and it is selectively expressed by B cell precursors. The association of μ_H and germline JCκ at the surface of

pre-B cells strongly suggests that the formation of pre-B receptors may not be exclusive to ψλL molecules. The relevance of ψLC during B cell development has been remarked by the conservation of these genes between the mouse and humans. Interestingly, such evolutionary conservation is shared by the transcription of germline JCκ (Leclerq *et al.*, 1989; Martin *et al.*, 1991; Thompson *et al.*, 1992), with equivalent potential in both species to initiate protein synthesis using non-canonic GUG codons (data not shown).

Whether such ψκL−μ association forms a functional receptor, either as an alternative pathway or as a back-up mechanism for the λ5 pre-B receptor, is currently uncertain. In support of a function for the surrogate germline κ product, previous reports have demonstrated that the induction of germline JCκ transcription, following activation of pre-B cell lines, correlates with the initiation of κ chain rearrangement (Schlissel and Baltimore, 1989; Shapiro *et al.*, 1993). Furthermore, the incomplete blockade of B cell precursor maturation in λ5-deficient mice (Kitamura *et al.*, 1992; Rolink *et al.*, 1993) would be in agreement with the notion that JCκ may be an alternative gene which functions similarly to λ5. Interestingly, analogous to λ5 gene targeting, deletion of the κ locus did not abrogate the production of Igλ-bearing B cells (Chen *et al.*, 1993; Zou *et al.*, 1993). Thus, germline JCκ would not be expected to be essential in B cell ontogeny, presumably because λ5 is intact in the κ-deficient mice. Taken together, these data are consistent with our working hypothesis that surrogate JCκ may form an alternative pre-B receptor. We propose (Figure 8) that during pre-B cell differentiation, μ_H chain can either associate with λ5 or germline JCκ to form a pre-B receptor. In this model, and as speculated for the ψλL−μ (Kitamura *et al.*, 1991, 1992; Tsubata *et al.*, 1992; Rolink *et al.*, 1993), the ψκL−μ pre-B receptor might transduce signals, resulting in L chain rearrangement and/or enhanced survival of productive V_HDJ_H cells that favour the emergence of mature B lymphocytes. Our model depicts λ5- and JCκ-associated receptors as differential pathways in B cell ontogeny. Alternatively, ψκL−μ may function as a back-up developmental pathway when the λ5/V pre-B receptor is defective or absent. Whether JCκ forms a pre-B receptor with μ_H in conjunction with a putative germline Vκ molecule, as suggested in Figure 8, or with Vpre-B in an analogous fashion to λ5, is a question which is currently under investigation.

Materials and methods

Southern blots

Genomic DNA was purified by standard procedures (Sambrook *et al.*, 1989). Following a *Hin*dIII digestion, 10 μg of DNA were electrophoresed on a 0.8% agarose gel, transferred onto a nylon membrane according to the protocol described by Southern (1975) and analysed by hybridization using either a Cκ or Cλ probe.

RNA isolation

Total RNA was isolated according to Chomczynski and Sacchi (1987) and purified into poly(A)$^+$, as reported previously (Davis *et al.*, 1986).

Reverse transcription

A total of 1 μg of total unfractionated RNA from B cell precursor ALL cells, normal B cell precursors or mature B cells was reverse-transcribed using the Superscript™ kit (Gibco-BRL, Gaithersburg, MD) according to the manufacturer's instructions.

PCR

A total of 5 μl of the reverse transcription reaction were amplified through 35 cycles (30 s at 94°C, 30 s at 60°C and 1 min at 72°C) using the method of Saiki *et al.* (1988).

cDNA construction and analysis

Generation and screening of the pre-ALP cDNA library was carried out using the λ-ZAP system (Stratagene, La Jolla, CA) according to the manufacturer's protocol. Sequencing was carried out by a modified version of the original method described by Sanger *et al.* (1977) using fluorescent primers (dye−primer reaction) or dNTPs (dye terminator), and an automated DNA sequencer (Applied Biosystems, Foster City, CA).

Translation of germline JCκ

In vitro transcription and translation were performed using commercial kits (Promega, Madison, WI) in the presence of [^{35}S]cysteine according to manufacturer's instructions. The translation products were analysed on a 12.5% SDS−PAGE gel as described by Laemmli (1970) under reducing conditions.

Isolation of normal B cell precursors, immunofluorescence and precipitation

Normal B cell precursors (CD10$^+$ sIg$^-$) were isolated from mid-term human fetal bone marrow, as described previously (Saeland *et al.*, 1993) and according to institutional guidelines. Fluorescence was analysed on a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA) employing standard techniques, using FITC-labelled mAbs. Histograms represent the log of fluorescence (*x* axis) versus the relative cell number (*y* axis). Cell membrane permeabilization was performed with 0.3% saponin (Sigma, St Louis, MO), before and during labelling. For immunoprecipitation, cells (10^7) were labelled with [^{35}S]cysteine for 3 h, followed by cell lysis and precipitation with indicated anti-κ antibodies. Labelled immunoprecipitates were resolved on a 12.5% SDS−PAGE, as described previously (Laemmli, 1970). Anti-JCκ serum was custom-made by The Neosystem Laboratory (Strasbourg, France). The anti-κ mAbs were obtained from Silenus Laboratories (Victoria, Australia) and Ortho Diagnostic Systems (Raritan, NJ).

Western blotting

Immunoblotting analysis was carried out following SDS−PAGE using a commercial semi-dry transfer system (Byolon, Dardilly, France) according to supplier's protocol. Reactivity to κ moieties was determined by the binding of the monoclonal anti-κ antibody (Silenus Laboratories) which recognizes both germline and rearranged κ molecules. The anti-κ reactivity was revealed by a 3 day exposure autoradiography, following a second ^{125}I-labelled anti-mouse antibody reaction.

2-D SDS−PAGE

Surface proteins were iodinated with ^{125}I by the lactoperoxidase technique (Hanstein, 1975) and immunoprecipitated with anti-κ mAb (Silenus Laboratories). For the first dimension, immunoprecipitates were run on a 10% SDS−PAGE gel without reduction. Subsequently, individual lanes were cut and immersed in 0.0625 M Tris, pH 6.8, containing 5% (v/v) 2-mercaptoethanol. Each gel slice was rotated by 90°, mounted on a separate 12.5% SDS−polyacrylamide gel and electrophoresed in the second dimension.

Acknowledgements

We thank Drs J.Banchereau and J.Chiller for support and advice on this project. We are grateful to Dr M.Cooper for kindly providing B207 cells, to Drs Y.J.Liu and S.Lebecque for critically reviewing the manuscript, to Dr T.LeBien for the generous gift of Blin-1, and to Mrs N.Courbière for editorial assistance. D.Pandrau-Garcia is a recipient of a fellowship from Fondation Mérieux (Lyon, France).

References

Alt,F., Rosenberg,N., Lewis,S., Thomas,E. and Baltimore,D. (1981) *Cell*, **27**, 381–390.

Alt,F.W., Oltz,E.M., Young,F., Groman,J., Taccioli,G. and Chen,J. (1992) *Immunol. Today*, **13**, 306–314.

Bernards,A. and de la Monte,S.M. (1990) *EMBO J.*, **9**, 2279–2287.
Bossy,D., Milili,M., Zucman,J., Thomas,G., Fougereau,M. and Schiff,C. (1991) *Int. Immunol.*, **3**, 1081–1090.
Bossy,D., Salamero,J., Olive,D., Fougereau,M. and Schiff,C. (1993) *Int. Immunol.*, **5**, 467–478.
Brouns,G.S., de Vries,E., van Noesel,C.J.M., Mason,D.Y., van Lier,R.A.W. and Borst,J. (1993) *Eur. J. Immunol.*, **23**, 1088–1097.
Chang,H., Dmitrovsky,E., Hieter,P.A., Mitchell,K., Leder,P., Turoczi,L., Kirsch,I.R. and Hollis,G.F. (1986) *J. Exp. Med.*, **163**, 425–435.
Chen,J., Trounstine,M., Kurahara,C., Young,F., Kuo,C.-C., Xu,Y., Loring,J.F., Alt,F.W. and Huszar,D. (1993) *EMBO J.*, **12**, 821–830.
Chomczynski,P. and Sacchi,N. (1987) *Anal. Biochem.*, **162**, 156–159.
Cooper,M.D. and Burrows,P.D. (1990) In Honjo,T., Alt,F.W. and Rabbits,T.H. (eds), *Immunoglobulin Genes*. Academic Press, San Diego, CA, pp. 1–21.
Davis,L.G., Dibner,M.D. and Battey,J.F. (1986) *Basic Methods in Molecular Biology*. Elsevier Science Publishers, New York.
Findley,H.W., Cooper,M.D., Kim,T.H., Alvarado,C. and Ragab,A.H. (1982) *Blood*, **60**, 1305–1309.
Hann,S.R., King,M.W., Bentley,D.L., Anderson,C.W. and Eisenman,R.N. (1988) *Cell*, **52**, 185–195.
Hanstein,D. (1975) *J. Immunol. Methods*, **7**, 25–28.
Hollis,G.F., Evans,R.J., Stafford-Hollis,J.M., Korsmeyer,S.J. and McKearn,J.P. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 5552–5556.
Hurwitz,R., Hozier,J., LeBien,T., Minowada,J., Gajl-Peczalska,K., Kubonishi,I. and Kersey,J. (1979) *Int. J. Cancer*, **23**, 174–180.
Karasuyama,H., Kudo,A. and Melchers,F. (1990) *J. Exp. Med.*, **172**, 969–972.
Kerr,W.G., Cooper,M.D., Feng,L., Burrows,P.D. and Hendershot,L.M. (1989) *Int. Immunol.*, **1**, 355–361.
Kitamura,D., Roes,J., Kühn,R. and Rajewsky,K. (1991) *Nature*, **350**, 423–426.
Kitamura,D., Kudo,A., Schaal,S., Müller,W., Melchers,F. and Rajewsky,K. (1992) *Cell*, **69**, 823–831.
Kudo,A. and Melchers,F. (1987) *EMBO J.*, **6**, 2267–2272.
Laemmli,U.K. (1970) *Nature*, **227**, 680–685.
Leclerq,L., Butkeraitis,P. and Reth,M. (1989) *Nucleic Acids Res.*, **17**, 6809–6819.
Martin,D., Huang,R., LeBien,T. and Van Ness,B. (1991) *J. Exp. Med.*, **173**, 639–645.
Misener,V., Downey,G.P. and Jongstra,J. (1991) *Int. Immunol.*, **3**, 1129–1136.
Pandrau,D., Francès,F., Martinez-Valdez,H., Pagès,M.-P., Manel,A.-M., Philippe,N., Banchereau,J. and Saeland,S. (1993) *Leukemia*, **7**, 635–642.
Pillai,S. and Baltimore,D. (1987) *Nature*, **329**, 172–176.
Pillai,S. and Baltimore,D. (1988) *Curr. Topics Microbiol. Immunol.*, **137**, 136–139.
Reth,M., Petrac,E., Wiese,P., Lobel,L. and Alt,F.W. (1987) *EMBO J.*, **6**, 3299–3305.
Rolink,A., Karasuyama,H., Grawunder,U., Haasner,D., Kudo,A. and Melchers,F. (1993) *Eur. J. Immunol.*, **23**, 1284–1288.
Saeland,S., Duvert,V., Moreau,I. and Banchereau,J. (1993) *J. Exp. Med.*, **178**, 113–120.
Saiki,R.K., Gelfand,D.H., Stoffel,S., Scharf,S.J., Higuchi,R., Horn,G.T., Mullis,K.B. and Erlich,H.A. (1988) *Science*, **239**, 487–491.
Sakaguchi,N. and Melchers,F. (1986) *Nature*, **324**, 579–582.
Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl Acad. Sci. USA*, **74**, 5463–5469.
Schlissel,M.S. and Baltimore,D. (1989) *Cell*, **58**, 1001–1007.
Shapiro,A.M., Schlissel,M.S., Baltimore,D. and DeFranco,A.L. (1993) *Mol. Cell. Biol.*, **13**, 5679–5690.
Southern,E.J. (1975) *J. Mol. Biol.*, **98**, 503–517.
Takemori,T. *et al.* (1990) *EMBO J.*, **9**, 2493–2500.
Thompson,A., Timmers,E., Kenter,M.J.H., Kraakman,M.E.M., Hendriks,R.W. and Schuurman,R.K.B. (1992) *Eur. J. Immunol.*, **22**, 3167–3171.
Tonegawa,S. (1983) *Nature*, **302**, 575–581.
Tsubata,T., Tsubata,R. and Reth,M. (1992) *Int. Immunol.*, **4**, 637–641.
Van Ness,B.G., Weigert,M., Coleclough,C., Mather,E.L., Kelly,D.E. and Perry,R.P. (1981) *Cell*, **27**, 593–598.
Zou,Y.-R., Takeda,S. and Rajewsky,K. (1993) *EMBO J.*, **12**, 811–820.

Received on August 4, 1994; revised on September 15, 1994