The EMBO Journal vol.7 no.11 pp.3433-3439, 1988

A second subunit of CD8 is expressed in human T cells

Anne M.Norment[1] and Dan R.Littman[1,2]

[1]Department of Microbiology and Immunology, and [2]Howard Hughes Medical Institute, University of California—San Francisco, San Francisco, CA 94143, USA

Communicated by A.F.Williams

The CD8 glycoprotein plays important functions in T cell development and in T cell activation. In rodents, CD8 is a heterodimer, consisting of an α-chain (Lyt2) and a β-chain (Lyt3). In humans, only the α-chain has been detected, and it has been thought that CD8 consists of homodimers of this protein. We have isolated functional cDNA clones encoding human CD8β, and show that the CD8β protein is expressed on the surface of CD8$^+$ human T cells. cDNA clones encoding multiple forms of the human CD8 β-chain have been isolated and characterized. These structural variants, which are likely to arise by alternative splicing, differ in the sequences encoding the cytoplasmic domain, which can consist of 19, 30 or 52 amino acids. One of the cDNAs lacks nucleotide sequences corresponding to a hydrophobic transmembrane domain, and may encode a secreted CD8β protein. The protein product of the human CD8β gene can be detected by a recently described anti-CD8 monoclonal antibody, 597. Expression of the epitope recognized by this antibody requires co-expression of the CD8α and CD8β gene products. About 90% of human CD8α positive thymocytes and peripheral blood lymphocytes express CD8β at the cell surface. Expression of the CD8β chain is thus conserved between human and rodents, and the variant CD8β polypeptides may have distinct roles in T cell function and development.

Key words: cell-cell interactions/CD8/Lyt3/T cell/alternative splicing

Introduction

The CD4 and CD8 glycoproteins are expressed on separate subpopulations of T lymphocytes and are thought to play major roles in thymocyte development and in interactions between T cells and their targets (reviewed by Littman, 1987). While the T cell antigen receptor (TCR) determines the antigen and MHC specificity of T cells, the expression of either the CD4 or CD8 glycoprotein is usually required for effective T cell activation (Dembíc *et al.*, 1987; Gabert *et al.*, 1987). A correlation exists between the expression of CD4 or CD8 and the major histocompatibility complex (MHC) specificity of a particular T cell. T cells that express CD4 respond to class II MHC molecules, while T cells that express CD8 respond to class I MHC molecules (Swain, 1983). Based on this correlation and on the observation that anti-CD4 and anti-CD8 antibodies inhibit T cell function, it was suggested that CD4 and CD8 increase the avidity of T cells for their targets by binding to class II or class I MHC molecules respectively (Marrack *et al.*, 1983; Swain, 1983). Low-affinity binding between CD4 and MHC class II molecules in the absence of the TCR has been recently demonstrated (Doyle and Strominger, 1987), and we have observed similar binding between CD8 and MHC class I molecules (Norment *et al.*, 1988).

In addition to their ability to enhance T cell-target cell binding, CD4 and CD8 appear to alter activation signals in mature T cells. In the absence of cell-cell binding, monoclonal antibodies against CD4 or CD8 will inhibit activation of T cells by anti-TCR antibodies (Moldwin *et al.*, 1987; Blue *et al.*, 1988). It is unclear whether this effect is due to a negative regulatory signal transmitted directly to T cells, or whether there is steric hindrance of TCR cross-linking by the presence of anti-CD4 or anti-CD8 antibodies (Owens and Fazekas de St Groth, 1987). In contrast, when anti-CD4 or anti-CD8 monoclonal antibodies are cross-linked to anti-TCR antibodies, a marked synergy in activation is observed (Emmrich *et al.*, 1987; Walker *et al.*, 1987). It has also been speculated that CD4 or CD8 may become physically associated with the TCR (Saizawa *et al.*, 1987; Takada and Engleman, 1987; Rivas *et al.*, 1988). The transmembrane domains and cytoplasmic tails of CD4 and CD8 are highly conserved between humans and rodents (Littman, 1987), suggesting that these regions may be involved in signal transduction upon contact of T cells with their targets.

Even though CD4 and CD8 are both members of the immunoglobulin (Ig) gene superfamily (Williams and Barclay, 1988), they do not share any sequence or structural similarities to suggest that they perform similar functions. CD4 is a 55-kd glycoprotein that appears to be expressed as a monomer. In humans, CD8 has been shown to be composed of homo-multimers of a 32- to 34-kd glycoprotein. In contrast, rodent CD8 is composed of two distinct chains, α (Lyt2) and β (Lyt3), which are encoded by closely linked genes (Gorman *et al.*, 1988). The α-chain corresponds to the human CD8 polypeptide; no human equivalent of the β-chain polypeptide has been detected, despite extensive biochemical analysis of CD8 (Snow and Terhorst, 1983; Snow *et al.*, 1984). Recently, a human CD8β partial genomic clone has been identified (Johnson, 1987). This clone contains an open reading frame that encodes a protein homologous to rodent CD8β. CD8β transcripts are present in thymocytes and in the human T cell tumor line HPB-ALL at levels comparable to those of CD8α (Johnson, 1987). This result prompted us to search for a CD8β protein in human T cells.

Here we report the isolation and expression of full-length cDNAs encoding human CD8β (Lyt3). The human CD8β cDNAs encode proteins that can be detected by a recently described anti-CD8 monoclonal antibody (mAb). Staining with this mAb demonstrates that the majority of CD8α-positive peripheral blood lymphocytes (PBL) also express

CD8β. In addition, multiple forms of CD8β cDNAs, most likely resulting from alternative splicing, have been identified. These cDNAs encode proteins having different cytoplasmic domains or lacking a transmembrane anchor sequence. These variant CD8 proteins may differ in their functions in cell–cell interactions and T cell activation.

Results

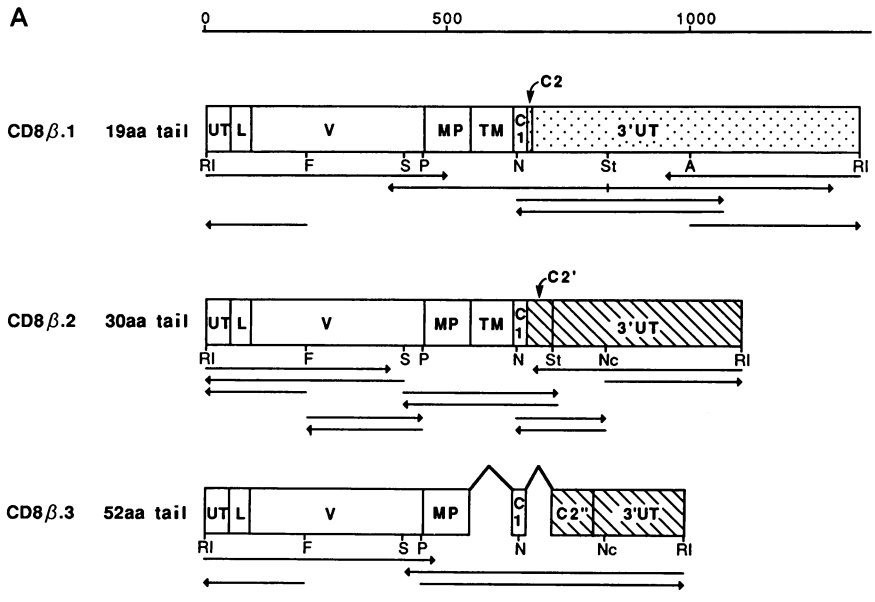
Cloning and sequence of a human CD8β cDNA

A partial human CD8β genomic clone containing exons encoding the Ig-like region, a membrane proximal domain, a putative transmembrane domain and the first part of the cytoplasmic tail has been previously isolated (Johnson, 1987). Sequences encoding the 5′ untranslated region, leader peptide and 3′ untranslated region were not identified, and it was possible that the gene or its product might be defective in humans. In order to establish whether human CD8β transcripts contain an open reading frame capable of encoding a functional protein, we have isolated and characterized human CD8β cDNAs. A λgt10 cDNA library prepared from human peripheral T cells was screened with a fragment from the Ig-like region of the human CD8β (Lyt3) genomic clone (Johnson, 1987). Three cDNA clones were isolated and sequenced. The restriction maps and nucleotide sequences of these clones are shown in Figure 1. The cDNAs are identical from the 5′ untranslated sequence, through an open reading frame that encodes a putative hydrophobic leader peptide, an Ig-like region and a serine- and threonine-rich membrane proximal domain. There is a single site for potential N-linked glycosylation at Asn-81. Beyond the membrane proximal domain, the three cDNAs diverge. A putative hydrophobic transmembrane domain is encoded by β.1 and β.2 but not by β.3 (Figure 1A and B). Based on a comparison with the sequence of genomic DNA (Johnson, 1987), it is clear that the absence of the transmembrane domain from β.3 is the result of an in-frame alternative splicing event that excludes the exon encoding nucleotides 543–633. This finding is of particular interest because similar splicing, which excludes nucleotides encoded by the transmembrane exon from CD8α transcripts, results in the production of a secreted CD8α homodimer (A.Norment and D.Littman, in preparation). The alternatively spliced forms of the two chains may therefore encode products that form a secreted heterodimer.

The differences in the putative cytoplasmic domains encoded by the three CD8β cDNAs also appear to be due to alternative splicing. The lengths of the variant cytoplasmic tails are indicated in Figure 1A. Codons in the transmembrane exon encode the first three amino acids of the cytoplasmic tail. All three cDNA clones have a nucleotide sequence (C1), defined by previous sequencing of genomic DNA, that encodes 13 cytoplasmic amino acid residues. Additional sequences not yet defined in genomic DNA are found 3′ to the C1 region in the three cDNAs. β.1 contains a sequence 3′ to C1 that encodes three additional amino acids. The predicted cytoplasmic domain of β.1 is most like that of rodent CD8β; it is the same size and contains 13/19 identities with the rat homolog (Johnson and Williams, 1986). In β.2, C1 is followed by a distinct sequence that encodes 14 additional amino acids (C2′). In β.3, the C2′ segment is absent, and C1 is followed by C2″, which encodes an additional 36 amino acids. This sequence is identical to the 3′ untranslated sequence of β.2; the transcript corresponding to CD8β.3 therefore probably arises by exclusion of an exon encoding C2′. Another less likely explanation is that this transcript results from the use of an alternate splice acceptor within the 3′ untranslated region of β.2; however, this putative splice acceptor lacks the consensus upstream pyrimidines (Mount, 1982). A search of the NBRF database failed to identify any known proteins with homology to C2′ and C2″. Although each cytoplasmic domain contains unique tyrosine and serine residues, none of these is predicted to be a substrate for tyrosine kinases (Hunter and Cooper, 1985) or protein kinase C (House *et al.*, 1987).

In vitro translation of human CD8β protein

To determine whether the human CD8β cDNA could encode a full-length membrane protein, RNA was synthesized and translated *in vitro* from the CD8β.2 cDNA (Figure 2). A translation product migrating with an apparent relative



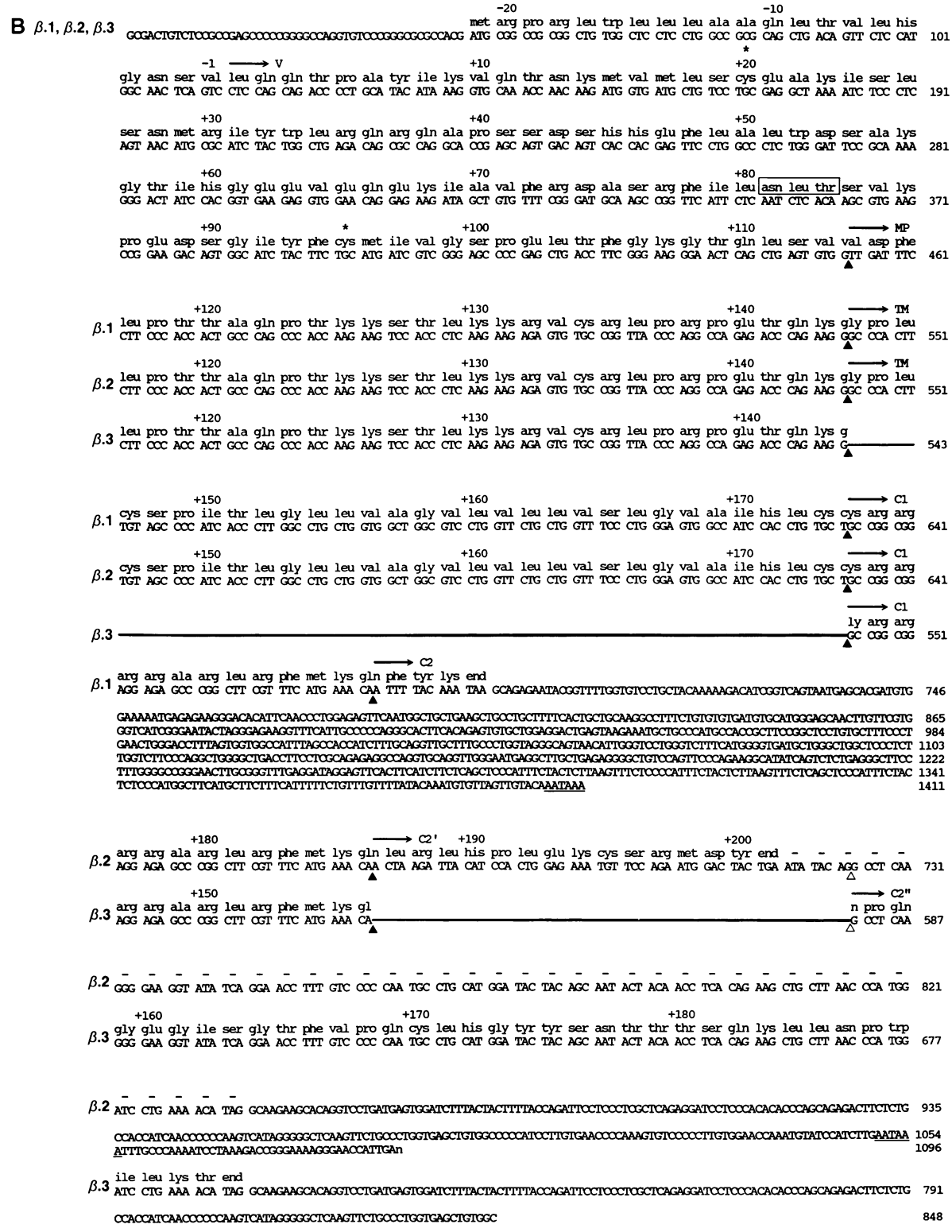


Fig. 1. Nucleotide sequence of human CD8β cDNAs and translated sequence of the corresponding proteins. Sequences encoding the 5′ untranslated (UT), leader (L), immunoglobulin V-like (V), membrane proximal (MP), transmembrane (TM), cytoplasmic (C) and 3′ untranslated (3′UT) regions are indicated. (**A**) Schematic representation of human CD8β cDNAs. A scale in nucleotide base pairs is indicated at the top of the figure. The region of CD8β.1 (stippled) that differs from CD8β.2 and CD8β.3 (stripes) is marked. Arrows represent fragments sequenced as described in Materials and methods. The length of the predicted cytoplasmic tail of each putative CD8β protein is indicated to the left of the figure. Restriction endonuclease sites are as follows: *Eco*RI (RI), *Fsp*I (F), *Sau*3AI (S), *Pvu*II (P), *Nae*I (N), *Stu*I (S), *Ava*II (A), *Nco*I (Nc). (**B**) Sequence of the CD8β cDNAs and the predicted proteins. The numbers to the right show nucleotide positions. Numbers shown above the amino acid sequence designate residues in the mature protein, as predicted by alignment with the rat CD8β (Lyt3) protein sequence (Johnson and Williams, 1986). Arrows designate domains of the proteins listed above. Solid lines indicate sequences absent in CD8β.3. Dashes indicate amino acid residues absent in CD8β.2. Two cysteines believed to form the Ig-like intrachain disulfide bond are marked by an asterisk above the amino acid sequence. A potential N-linked glycosylation site at Asn-81 is boxed. Solid arrowheads indicate known intron−exon boundaries (Johnson, 1987); open arrowheads indicate potential intron−exon boundaries predicted by the sequences of the cDNAs. Polyadenylation signals are underlined.

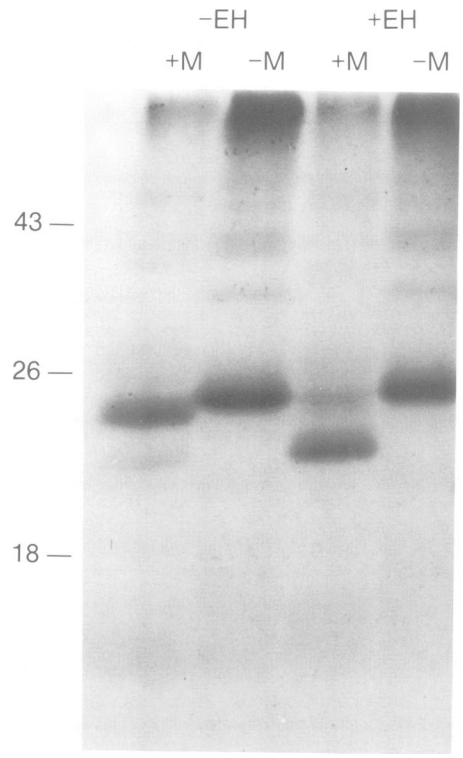


Fig. 2. *In vitro* translation of CD8β.2. The human CD8β.2 cDNA was transcribed *in vitro*, and the RNA was then translated using rabbit reticulocyte lysates in the presence (+M) or in the absence (−M) of microsomal membranes. Samples were then incubated in the presence (+EH) or in the absence (−EH) of endoglycosidase H, and analyzed by SDS−PAGE. Relative molecular masses (M_r) are given in kilodaltons to the left of the figure.

molecular mass of 25 kd was observed by SDS−PAGE. Translation of mRNA derived from the CD8β.2 cDNA in the presence of microsomal membranes resulted in a 24-kd protein that was decreased in size to 22.5 kd following incubation with endoglycosidase H (Figure 2). These results are consistent with the size of the precursor protein (25 236 daltons) and of the cleavage product (22 856) predicted from the primary sequence, assuming that the cleavage of the leader sequence is at a position corresponding to that in rat CD8β (Johnson and Williams, 1986). With a single predicted N-linked glycosylation site, the molecular mass of the mature CD8β.1 and CD8β.2 proteins would be ~25 and ~26 kd respectively. However as in CD8α, there may be additional O-linked glycosylation of the serine- and threonine-rich membrane proximal domain (Snow *et al.*, 1985). The sizes of mature CD8α and CD8β glycoproteins may thus be very similar.

Expression of human CD8β transcripts

To assess the expression of the CD8β gene products, L cells were transfected with expression vectors encoding CD8β.2, CD8α or both (Figure 3). RNA samples prepared from transfected cell lines, from human tissues and from human T cell leukemias were analyzed for the presence of CD8α and CD8β transcripts. As shown in Figure 3, transfected cells expressed a 2.3-kb CD8α transcript and a 1.5-kb CD8β transcript at levels comparable to those of the T cell leukemia line HPB-ALL. A 1.5-kb CD8β mRNA and a minor transcript of 1.0 kb are expressed in human thymocytes (THY), lymph node cells (LN) and spleen cells (SPL)

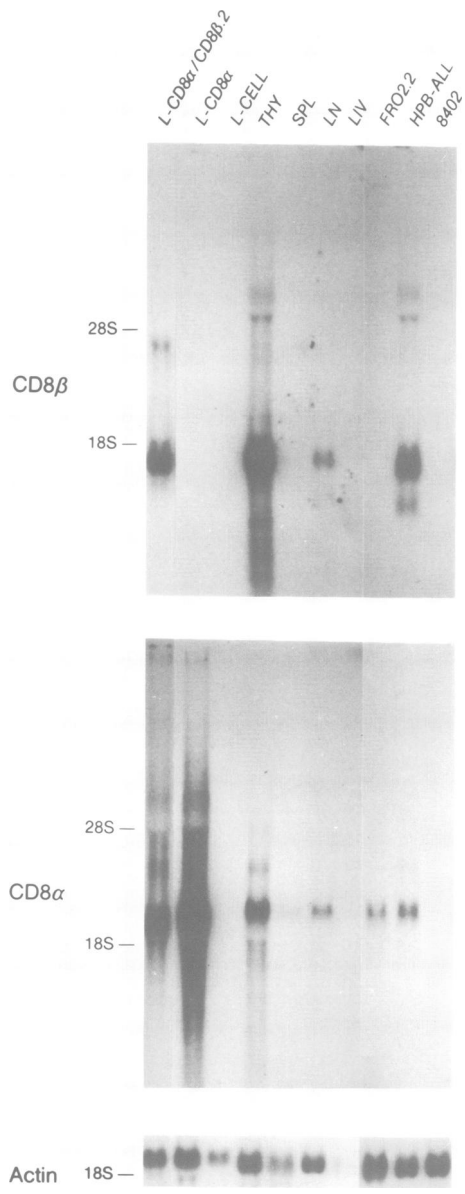


Fig. 3. Northern blot analysis of human CD8 transcripts. Total RNA from L cells, L cells transfected with CD8α or CD8α/CD8β, normal human tissues, and T cell leukemias was analyzed for expression of CD8β, CD8α, and actin transcripts as described in Materials and methods. Normal human tissues include: thymus (THY), spleen (SPL), lymph node (LN) and liver (LIV). T cell leukemia lines include: FRO2.2 ($CD4^+$, $CD8^+$), HPB-ALL ($CD4^+$, $CD8^+$) and 8402 ($CD4^-$, $CD8^-$). The position of 18S and 28S rRNAs is indicated to the left of the figure.

(Figure 3). Although both HPB-ALL and FRO2.2 express CD8α mRNA, only HPB-ALL expresses the CD8β transcript. The T cell leukemia line, 8402, fails to express either CD8 transcript.

Detection of the human CD8β protein

MAbs reactive with human CD8 have been shown to differ in their ability to bind to peripheral T cells and to CD8α-transfected L cells (Martin *et al.*, 1984; Disanto *et al.*, 1987). These observations suggested that some of these antibodies may recognize epitopes of CD8β or combined epitopes of CD8α and CD8β. A panel of anti-CD8 mAb (Disanto *et al.*, 1987) was therefore screened for reactivity with CD8α-

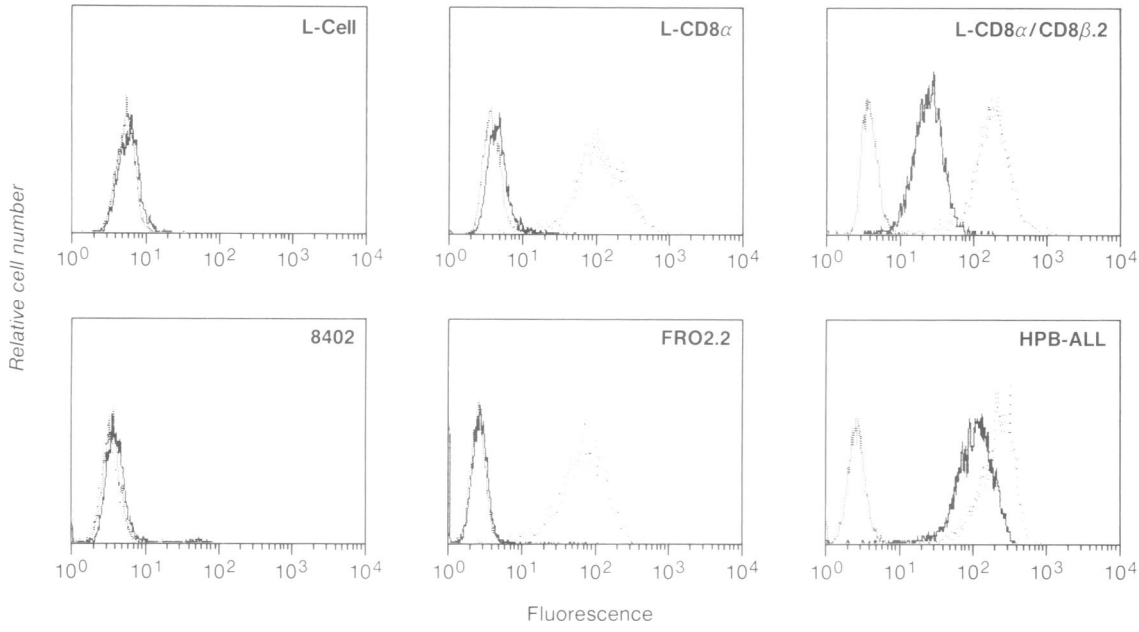


Fig. 4. Surface immunofluorescence analysis of human CD8 expression by transfected cells and T cell lines. Cells are described in Materials and methods, and the legend to Figure 3. Cells were incubated with the anti-CD8α monoclonal antibody OKT8 (fine dotted line), 597 (solid line) or control antibody (heavy dotted line). The control antibodies were W6/32 (anti-MHC class I), for L-tk$^-$ cells and their derivatives, and GK1.5 (anti-murine CD4), for the T cell leukemia lines. Staining utilized a second step fluorescein-conjugated goat anti-mouse IgG antibody. Fluorescence is marked in arbitrary units along the *x*-axis.

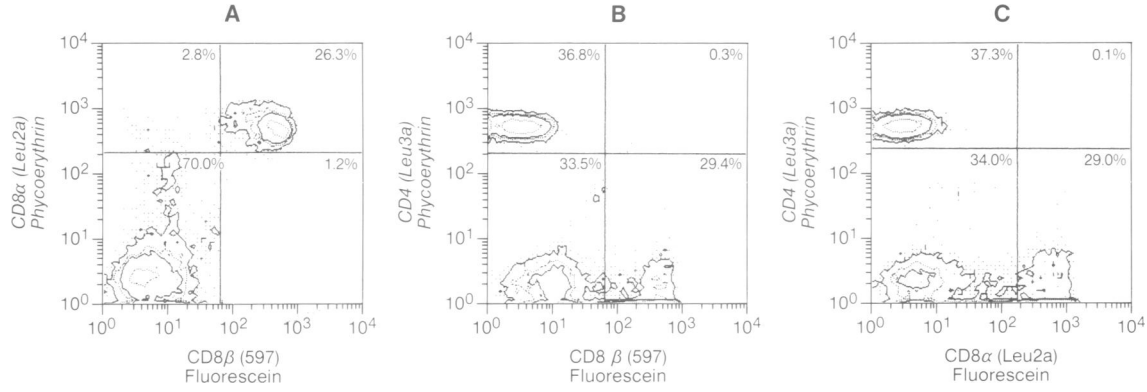


Fig. 5. Dual-color immunofluorescence analysis of human CD8 on PBL. Human PBL were stained as described in Materials and methods using the monoclonal antibodies indicated along the *x*- and *y*-axes of the figure. Fluorescence is marked in arbitrary units along the axes. Statistical markers were set to exclude the dim CD8α$^+$ cells; the percentage of gated lymphocytes is indicated in the upper right-hand corner of each quadrant. None of the dim CD8α$^+$ cells expressed the 597 determinant.

positive L cells by surface immunofluorescence. Of 40 antibodies tested, 38 bound well to the surface of transfected cells, one (572) bound poorly and another (597) did not bind at all. The mAb 597 has been shown previously not to bind to CD8α-transfected cells (Disanto *et al.*, 1987). When mAbs 572 and 597 were tested for binding to L cells transfected with both of the CD8α and CD8β cDNAs, 597 reacted with the cells (Figure 4). In addition, 597 reacts with HPB-ALL cells, which express mRNA for both CD8 chains, but it does not react with FRO2.2 cells, which express CD8α alone. MAb 597 does not bind to cells transfected with CD8β.2 in the absence of CD8α (data not shown). These results indicate that mAb 597 detects a product of the CD8β gene that is expressed on the surface of transfected cells and on the human T cell leukemia HPB-ALL.

The expression of the 597 epitope on normal human PBL and thymocytes was examined by two-color surface immunofluorescence. About 90% of bright CD8α-positive PBL stain with 597 (Figure 5, panel A); in contrast, none of the CD4 positive cells stain with 597 (panel B). In the same experiment, 23% of dim CD8α$^+$ cells specifically bound the natural killer cell specific mAb leu11 (Lanier *et al.*, 1983), but none bound mAb 597 (data not shown). This observation suggests that unlike CD8α, CD8β is not expressed on the human natural killer cell population. Alternatively, a threshold level of CD8α expression may be required for

expression of CD8β at the cell surface. Of human thymocytes, ~90% of CD8α-positive cells express the 597 epitope. Expression of CD8β is similar for $CD4^+/CD8^+$ double-positive thymocytes and mature $CD4^-/CD8^+$ single-positive thymocytes (data not shown). We were unable to detect a population of PBL or thymocytes which express the 597 epitope in the absence of CD8α.

It appears that CD8β-positive T cells may be a subset of CD8α-positive T cells. In mouse, surface expression of the CD8β-chain (Lyt3) is thought to be dependent on expression of the CD8α (Lyt2) subunit (Blanc *et al.*, 1988; Gorman *et al.*, 1988). In our studies, CD8β could be detected by mAb 597 only on cells that also expressed CD8α. The possibility remains that human CD8β can also be expressed at the surface in the absence of CD8α; it has not been determined whether the epitope recognized by mAb 597 resides on the CD8β chain or is specified by both chains.

Discussion

These studies indicate that the human CD8β gene product is expressed on the surface of the $CD8^+$ subset of T lymphocytes. Based on these findings, it is puzzling that the human CD8β protein has not been previously detected. For example, proteolytic analysis of CD8 immunoprecipitated from ^{125}I-surface-labeled HPB-ALL cells and PBL indicates that human CD8 is composed of the single CD8α chain (Snow and Terhorst, 1983). This was confirmed by amino-terminal sequencing of CD8 isolated from HPB-ALL cells (Snow *et al.*, 1984). Several possibilities may account for this paradox: (i) CD8β may co-migrate with CD8α when analyzed by SDS−PAGE; (ii) CD8β may label poorly with ^{125}I; (iii) there may be variations in CD8β expression by leukemic cell lines analyzed in different laboratories; (iv) the amino terminus of the CD8β chain from HPB-ALL cells may be blocked; (v) the anti-CD8 mAbs used for immunoprecipitation or purification may react with the CD8α homodimer, but not with the heterodimer; and (vi) unlike rodent CD8β, the human homolog may not be disulfide linked to CD8α, and association of the two chains may be disrupted upon detergent solubilization. When mAb 597 was used to immunoprecipitate lysates of ^{125}I-surface-labeled PBL, no protein was detected (Disanto *et al.*, 1987). However, we have been able to detect a small amount of protein by immunoprecipitation of [^{35}S]cysteine-labeled lysates of HPB-ALL cells using mAb 597. This co-migrates with CD8α under reducing and non-reducing conditions (data not shown).

The three cDNAs that we have isolated encode CD8β polypeptides that differ in their cytoplasmic domains. This is interesting in light of recent evidence that the CD4 and CD8 glycoproteins may function not only in adhesion, but also in transduction of regulatory signals in the course of T cell activation (Emmrich *et al.*, 1987). The variant CD8β polypeptides may differ in their abilities to interact with components of the cytoskeleton and with other T cell surface proteins, or they may be subject to alternative means of regulation through covalent modification. These forms of CD8β may also have different requirements for association with CD8α and surface expression.

The structure and pattern of expression of CD8β appear to be conserved between rodents and humans, suggesting that this molecule serves an important function. Gene transfer experiments indicate that CD8α and the α- and β-TCR genes are sufficient to reconstitute antigen-specific T cell activation (Gabert *et al.*, 1987). It is unclear whether CD8β would have any function in this system. We have detected binding of CD8α to class I MHC molecules in the absence of the T cell antigen receptor (Norment *et al.*, 1988). It is possible that CD8α homo-multimers may be sufficient for binding to some MHC class I molecules, but that αβ heterodimers may extend the range of polymorphic class I molecules to which CD8 can bind. Alternatively CD8β may be primarily involved in transmission of regulatory signals to T cells upon binding of CD8α to class I MHC molecules on the target cell.

Materials and methods

Library screening and DNA sequencing

Preparation of a λgt10 human peripheral T cell cDNA library was as described (Littman *et al.*, 1985). By hybridization with a nick-translated (Bethesda Research Laboratory) CD8β genomic *Nco*I−*Pvu*II fragment (a gift of Drs P.Johnson and A.Williams, University of Oxford), 11 positive plaques were identified out of 6×10^5 screened. Hybridization was performed overnight in 30% formamide, 5 × SSCPE and 5 × Denhart's solution. Washes were at 55°C in 0.1% SDS and 2 × SSC. Three plaques were isolated, and the insert DNA subcloned into M13mp18 (New England Biolabs) or Bluescript M13-KS (Stratagene) at the *Eco*RI site. Sequencing was performed on both strands of single-stranded DNA by dideoxy-chain termination (Sanger *et al.*, 1977).

In vitro transcription and translation

The CD8β.2 cDNA was subcloned into the vector pSP72 (Krieg and Melton, 1987), linearized with *Hin*dIII, and transcribed according to standard protocols with T7 polymerase. *In vitro* translation was subsequently performed using rabbit reticulocyte lysates (Promega) according to the manufacturer's instructions, with [^{35}S]methionine (>600 Ci/mmol, Amersham) included in the reaction, in the presence or absence of canine pancreatic microsomal membranes [a gift of Drs V.Lingappa and D.W. Andrews, University of California—San Francisco (UCSF)] for 60 min at 26°C. Endoglycosidase H (Boehringer Mannheim) digestion was performed overnight at 37°C in the presence of 0.02 U/ml of enzyme, 0.1 M sodium citrate, pH 5.5, 0.1% SDS. Control samples received no endoglycosidase H. Samples were analyzed by SDS−PAGE on a 12.5% Laemmli gel.

Transfection of L-tk$^-$ cells

Murine L cells deficient in thymidine kinase (L-tk$^-$) were transfected by calcium phosphate precipitation with the CD8α cDNA pT8F1 (Littman *et al.*, 1985) inserted into the vector pMV6Tkneo (Maddon *et al.*, 1985), and selected in 400 μg/ml of G418 (Geneticin, Gibco). Colonies expressing CD8α were detected by rosetting using the monoclonal antibody OKT8, and human red blood cells conjugated with rabbit anti-mouse IgG (Zymed) as described (Littman *et al.*, 1985). One $CD8\alpha^+$ clone (L-CD8α) and L-tk$^-$ cells were subsequently co-transfected with pTk, and the CD8β.2 cDNA subcloned into the vector pSV7d (from Dr P.Luciw, University of California—Davis), a derivative of pHS210 (Stuvé *et al.*, 1987). After selection in hypoxanthine, aminopterin and thymidine (HAT), colonies were expanded and analyzed for expression of CD8β mRNA on Northern blots and a representative colony was chosen for further analysis.

Cells

Murine L cells (tk$^-$) were maintained in Dulbecco's modified Eagle's medium (DME) supplemented with 10% fetal bovine serum (FBS, Gibco). Human T cell leukemia lines FRO2.2, 8402, and HPB-ALL (Littman *et al.*, 1985) were grown in RPMI 1640 supplemented with 12.5% FBS (Hyclone). Human PBL were isolated by Ficoll-Hypaque density gradient centrifugation. Human thymocytes (from Dr L.Bockenstedt, UCSF) were isolated from fresh tissue samples, and frozen with liquid nitrogen in RPMI 1640, 10% FBS and 10% DMSO. Aliquots of human thymocytes were thawed in the presence of 1 μg/ml DNase I.

Northern blots

Eight micrograms of total RNA was fractionated on a 1% agarose gel containing 40 mM morpholinopropanesulfonic acid (MOPS), 10 mM sodium

acetate and 1 mM EDTA plus 7% formaldehyde, and transferred to Genescreen (New England Nuclear) in 20 × SSC. RNA was crosslinked to the filter by UV irradiation. Hybridization was performed according to Church and Gilbert (1984) in 0.5 M sodium phosphate, pH 7.2, 7% SDS, 1% bovine serum albumin (BSA), 1 mM EDTA and 25% formamide for 24 h at 65°C with 10 ng/ml of nick-translated (Bethesda Research Laboratory) probe. The filter was washed in 40 mM sodium phosphate, pH 7.2, 1% SDS and 1 mM EDTA. The filter was first probed with the CD8β.2 cDNA (2×10^8 c.p.m./μg), and then stripped and reprobed with the CD8α cDNA T8F1 (Littman *et al.*, 1985), followed by a hamster actin cDNA as a control for RNA loading and degradation.

Antibodies and immunofluorescence analysis

Antibodies included in these studies were as follows. The panel of anti-CD8 antibodies was provided by the Third International Leukocyte Typing Workshop, Dr A.McMichael, director (Disanto *et al.*, 1987), and included the antibody 597 (2ST85H7). These antibodies were in the form of ascites and were diluted 1:100 for immunofluorescence studies. Additional 597 (IgG2a) was a gift of Dr E.Reinherz, and was used at a dilution of 1:200. OKT8 (IgG2a) and W6/32 (a gift of Dr P.Parham, Stanford University) were affinity purified by protein A sepharose chromatography and used at 10 μg/ml. GK1.5, Leu2a (unconjugated and phycoerythrin conjugated), Leu3a (phycoerythrin conjugated) and goat anti-mouse IgG (fluorescein conjugated) were from Becton Dickinson and were used according to the manufacturer's instructions. For immunofluorescence studies, 1×10^6 cells were incubated with saturating levels of antibody in 0.1 ml of PBS and 5% FBS for 30 min at 4°C. Cells were washed twice with 3 ml of 5% FBS in PBS at 4°C. For dual color immunofluorescence analysis, PBL and thymocytes were stained as follows: cells were incubated with 597 or Leu2a, and then with fluorescein-conjugated goat anti-mouse IgG; this was followed by blocking with saturating levels of normal mouse serum (Zymed) and staining with phycoerythrin-conjugated Leu2a or Leu3a antibody. Fluorescence was measured in arbitrary units using a FACScan cell analyzer (Becton Dickinson).

Acknowledgements

We thank P.Johnson and A.Williams for the genomic CD8β fragment; A.McMichael for the panel of anti-CD8 mAbs; E.Reinherz for 597 ascites; S.Stuart for the homology search of the NBRF database; and F.Brodsky, N.Landau and W.Seaman for critical reading of the manuscript. This research was supported by a grant from the NIH (D.R.L.), a Searle Scholar award (D.R.L.) and a Medical Scientist Training Program Fellowship (A.M.N.).

References

Blanc,D., Bron,C., Gabert,J., Letourneur,F., MacDonald,H.R. and Malissen,B. (1988) *Eur. J. Immunol.*, **18**, 613–619.

Blue,M.L., Hafler,D.A., Daley,J.F., Levine,H., Craig,K.A., Breitmeyer,J.B. and Schlossman,S.F. (1988) *J. Immunol.*, **140**, 376–383.

Church,G.M. and Gilbert,W. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1991–1995.

Dembíc,Z., Haas,W., Zamoyska,R., Parnes,J., Steinmetz,M. and von Boehmer,H. (1987) *Nature*, **326**, 510–512.

Disanto,J.P., Small,T.N., Dupont,B., Flomenberg,N. and Knowles,R.W. (1987) In McMichael,A.J. (ed.), *Leucocyte Typing III, White Cell Differentiation Antigens*. Oxford University Press, Oxford, pp. 210–214.

Doyle,C. and Strominger,J.L. (1987) *Nature*, **330**, 256–259.

Emmrich,F., Strittmatter,U. and Eichmann,K. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 8298–8302.

Emmrich,F., Kanz,L. and Eichmann,K. (1987) *Eur. J. Immunol.*, **17**, 529–534.

Gabert,J., Langlet,C., Zamoyska,R., Parnes,J.R., Schmitt-Verhulst,A.M. and Malissen,B. (1987) *Cell*, **50**, 545–554.

Gorman,S.D., Sun,Y.H., Zamoyska,R. and Parnes,J.R. (1988) *J. Immunol.*, **140**, 3646–3653.

House,C., Wettenhall,R.E.H. and Kemp,B.E. (1987) *J. Biol. Chem.*, **262**, 772–777.

Hunter,T. and Cooper,J.A. (1985) *Annu. Rev. Biochem.*, **54**, 897–930.

Johnson,P. (1987) *Immunogenetics*, **26**, 174–177.

Johnson,P. and Williams,A.F. (1986) *Nature*, **323**, 74–76.

Krieg,P.A. and Melton,D.A. (1987) *Methods Enzymol.*, **155**, 397–415.

Lanier,L., Le,A.M., Phillips,J.H., Warner,N.L. and Babcock,G.F. (1983) *J. Immunol.*, **131**, 1789–1796.

Ledbetter,J.A., June,C.H., Grosmaire,L.S. and Rabinovitch,P.S. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 1384–1388.

Littman,D.R. (1987) *Annu. Rev. Immunol.*, **5**, 561–584.

Littman,D.R., Thomas,Y., Maddon,P.J., Chess,L. and Axel,R. (1985) *Cell*, **40**, 237–246.

Maddon,P.J., Littman,D.R., Godfrey,M., Maddon,D.E., Chess,L. and Axel,R. (1985) *Cell*, **42**, 93–104.

Marrack,P., Endres,R., Shimonkevitz,R., Zlotnik,A., Dialynas,D., Fitch,F. and Kappler,J. (1983) *J. Exp. Med.*, **158**, 1077–1091.

Martin,P.J., LedbetterJ.A., Clark,E.A., Beatty,P.G. and Hansen,J.A. (1984) *J. Immunol.*, **132**, 759–765.

Moldwin,R.L., Havran,W.L., Nau,G., Lancki,D.W., Kim,D.K. and Fitch,F.W. (1987) *J. Immunol.*, **139**, 657–664.

Mount,S.M. (1982) *Nucleic Acids Res.*, **10**, 459–472.

Norment,A.N., Salter,R.D., Parham,P., Engelhard,V.H. and Littman,D.R. (1988) *Nature*, in press.

Owens,T. and Fazekas de St. Groth,B. (1987) *J. Immunol.*, **138**, 2402–2409.

Rivas,A., Takada,S., Koide,J., Sonderstrup-McDevitt,G. and Engleman, E.G. (1988) *J. Immunol.*, **140**, 2912–2918.

Saĭzawa,K., Rojo,J. and Janeway,C.A.,Jr (1987) *Nature*, **328**, 260–263.

Sanger,F., Nicklen,S. and Coulson,A. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.

Snow,P.M. and Terhorst,C. (1983) *J. Biol. Chem.*, **258**, 14675–14681.

Snow,P.M., Keizer,G., Coligan,J.E. and Terhorst,C. (1984) *J. Immunol.*, **133**, 2058–2066.

Snow,P.M., Coligan,J.E. and Terhorst,C. (1985) *J. Biol. Chem.*, **260**, 2700–2708.

Stuvé,L.L., Brown-Shimer,S., Pachl,C., Najarian,R., Dina,D. and Burke, R.L. (1987) *J. Virol.*, **61**, 326–335.

Swain,S.L. (1983) *Immunol. Rev.*, **74**, 129–142.

Takada,S. and Engleman,E.G. (1987) *J. Immunol.*, **139**, 3231–3235.

Walker,C., Bettens,F. and Pichler,W.J. (1987) *Eur. J. Immunol.*, **17**, 873–880.

Williams,A.F. and Barclay,A.N. (1988) *Annu. Rev. Immunol.*, **6**, 381–405.

Received on July 25, 1988