A SECOND CHAIN OF HUMAN CD8 IS EXPRESSED ON PERIPHERAL BLOOD LYMPHOCYTES

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CD8 is a T lymphocyte surface glycoprotein expressed on most thymocytes and on mature T cells that recognize or are restricted by class I MHC molecules (1). Most such cells are cytotoxic or suppressor in function. A large body of evidence now supports the hypothesis that the function of CD8 is to increase the avidity of the interaction between the T cell and target or APC and to enhance T cell activation, most likely by binding to a nonpolymorphic (or relatively nonpolymorphic) region of class I MHC proteins (1–7). Biochemical and molecular genetic studies have demonstrated that mouse (Ly-2,3) and rat (OX-8) CD8 consist of heterodimers (and higher multimers) of two distinct polypeptides (α and β) encoded by separate genes (8–14). In contrast, human CD8 has been consistently described on mature T cells as a homodimer or homomultimer of a single polypeptide chain (15, 16) homologous to mouse Ly-2α or rat OX-8 32K (CD8α) (10, 11, 13). This apparent difference in subunit composition is surprising for a molecule whose cellular distribution and functional role is so well conserved among these three species. A human gene homologous to mouse Ly-3 and rat OX-8 37K (CD8β) chains was recently identified and partially sequenced (17). mRNA corresponding to this gene was shown to be present in human thymus as well as a human leukemic T cell line (HPB-ALL), but the limited data did not allow any conclusions as to whether a protein could be expressed (17). We now report the isolation of cDNA clones encoding a second chain (CD8β) of the human CD8 antigen. Using the mAb 2ST8-5H7, we show expression of the encoded protein on L cell transfectants and on normal human peripheral blood cells in conjunction with the CD8α chain. Its expression is limited to $OKT8^+$ cells, suggesting that human CD8, like its mouse and rat counterparts, also exists as a heterodimer.

Materials and Methods

cDNA Cloning and Sequencing. A rat CD8β cDNA clone (pX9.5, reference 14) was labeled with ^{32}P by random hexamer priming (18) and used to screen a human thymocyte cDNA library in the vector λgt10. Filters were hybridized as described (10) and washed (30 min each) twice in 2× SSC, 0.05% SDS, at room temperature, once in 1× SSC, 0.05% SDS at 37°C, and twice in 0.5× SSC, 0.05% SDS at 42°C. Six hybridizing clones were selected based on insert size (1.3 kb or larger), subcloned into M13, and sequenced using the Sequenase system

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(United States Biochemical Corp., Cleveland, OH). Both strands of all six clones were completely sequenced. The genomic subclone pλ4.3 (17) was similarly subcloned and sequenced.

Northern Blotting Analysis. Total RNA from cell lines, tissue, or PBMC was isolated by the procedure of Chirgwin et al. (19). 10 μg of each sample was subjected to electrophoresis through a 1.5% agarose gel containing 2.2 M formaldehyde. The RNA was transferred to Genetran 45 (Plasco) and hybridized to a human CD8β cDNA fragment labeled with ^{32}P by random hexamer priming (18). Hybridization was performed at 42°C in 3× SSC, 2% SDS, 5× Denhardt's solution, 0.02 M sodium phosphate, 1 mM pyrophosphate, 100 μg/ml denatured herring sperm DNA, 100 μg poly C, and 50% formamide. Blots were washed as described (10).

Construction and Transfection of pSFSVn-HCD8. An expression vector containing the sequence encoding human CD8β was constructed by isolating the Eco RI–Rsa I fragment of a cDNA clone that contained the entire coding region and most of the 3′ untranslated region of human CD8β. The Eco RI end was blunt ended and the resulting 1.37-kb fragment subcloned into the blunt-ended Xho I site of the pSFSVneo vector (20). A clone in the orientation allowing transcription of sense mRNA was selected and designated pSFSVn-HCD8β. 10^6 thymidine kinase (tk)[1] deficient L cells were cotransfected as described (21) with 1 μg of pSFSVn-HCD8β and 20 μg of pBR322/TK/CD8α (a vector containing the human *CD8α* gene, reference 12) or 1 μg of pSFSVn-HCD8β and 20 μg of tk^- L cell carrier DNA. Transfectants were selected by growth in 300 μg/ml G418 (Gibco Laboratories, Grand Island, NY) for 2–3 wk and then analyzed with the FACS.

Cells and Culture Conditions. Peripheral blood from a healthy donor was mixed with an equal volume of RPMI containing 10% FCS and separated on Histopaque-1077 (Sigma Chemical Co., St. Louis, MO). The mononuclear cells were recovered, washed repeatedly to remove platelets, and resuspended for staining. L cell transfectants were cultured in high glucose DME (Irvine Scientific, Santa Ana, CA) containing 5% FCS and the appropriate selective medium. The HPB-ALL cell line was cultured in RPMI 1640 (Irvine Scientific) supplemented with 10% FCS.

Immunofluorescence and Flow Cytometry. Antibody stainings were performed sequentially in PBS with 1% BSA and 0.02% sodium azide. Flow cytometry and data analysis were performed using either a single or dual laser-modified FACS (FACS II; Becton Dickinson & Co., Mountain View, CA).

Results

Isolation of Human CD8β cDNA Clones. We screened a human thymocyte cDNA library with a cDNA probe corresponding to rat CD8β (14) and determined the nucleotide sequence of six of the isolated clones. Each contained a single open reading frame. The nucleotide and predicted amino acid sequences corresponding to four of these clones are shown in Fig. 1. The sequences predict a mature protein of 189 amino acids (predicted mol wt, 21,353), with an external domain of 143 amino acids, a hydrophobic transmembrane region of 27 amino acids, and a highly basic cytoplasmic tail of 19 amino acids. There is one potential *N*-linked glycosylation site at amino acid 81. The mature protein is preceded by a 21-amino acid signal peptide. As shown in Fig. 2, the amino acid sequence of mature human CD8β is 57% identical to both mouse (12, 22, 23) and rat (14) CD8β. The sequence is not closely related to that of human CD8α, with only 25% identical residues with numerous gaps in the alignment (data not shown). As has been previously observed (12, 14, 17, 22, 23), CD8β is a member of the Ig gene superfamily and contains NH_2-terminal domains homologous to Ig variable (V) regions and joining (J) segments. These Ig-like regions are connected to the membrane by a 29-amino acid connecting peptide

[1] *Abbreviation used in this paper:* tk, thymidine kinase.

FIGURE 1. Nucleotide and predicted amino acid sequence of thymocyte cDNA clones encoding human CD8β. Sequence corresponding to four human cDNA clones analogous to mouse and rat CD8β is shown. Allelic differences found in two of the cDNA clones represented here are indicated below the sequences found in the other two clones and are located at positions 583, 1281, and 1326 in the nucleotide sequence. Horizontal arrows indicate the beginning of the predicted leader (*L*) or signal peptide, V-like domain (*V*), J-like segment (*J*), connecting peptide (*CP*), transmembrane region (*TM*), and cytoplasmic domain (*CY*). The start site of the mature protein was determined by comparison with the NH_2-terminal protein sequence of mouse CD8β (37). A closed circle indicates the potential *N*-linked glycosylation site, and asterisks mark cysteine residues. The polyadenylation signal is underlined. Polyadenylation begins at nucleotide 1398 in each of the cDNA clones containing a poly(A) tail. The numbers of the first and last amino acid and nucleotide in each line are indicated in the left and right margins, respectively. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00805.

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                  ⊢→L                    ⊢→V
Human    -21  MRPRLWLLLAAQLTVLHGNSVLQQTPAYIKVQTNKMVMLSCEAK-ISLSNMRIYWLRQR   37
Mouse    -21  *Q*W***VFSMK*AA*WSS*A*I***SSLL****HTAKM***V*S**-KLTS*****E*   37
Rat      -21  *Q*W***VFSVK*SA*W*S*A*L***SSLL****QTAKN*****-TFPKGTT*****EL   37

Human     38  QAPSSDSHHEFLALWDSAKGTIHGEEVEQEK-IAVFRDASR-FILNLTSVKPEDSGIYF   94
Mouse     38  *D*-K*KYF****S*S*S**VLY**S*DKKRN*ILESSD**RPF*SIMN******DF**   95
Rat       38  *DSNKNK*F****SRT*T**IKY**R*-KK-NMTL-SFN*TLPF*KIMD*******F**   93

                  ⊢→J      ⊢→CP                              ⊢→TM
Human     95  CMIVGSPDLTFGKGTQLSVVDFLPTTAQPTKKSTLK---KRVCRLPRPETQKGPLCSPI  150
Mouse     96  *AT****KMV**T**K*T***V*****-****T***MKK*KQ*PF*H******LT**LT  153
Rat       94  *AM****MVV**T**K*T***V*****-****T******---*KQ*PT*H*K****LT*GL*  149

                        ⊢→CY
Human    151  TLGLLVAGVLVLLVSLGVAIHLCCRRRRARLRFMKQFYK                       189
Mouse    154  **S***VCI*L**AF****VYFY*V*****IH*****H*                       192
Rat      150  **S****C********S****FH*M******IH******H*                     187
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FIGURE 2. Comparison of predicted amino acid sequences of human, mouse, and rat CD8β. Amino acids are designated by the one letter code. Identical residues are indicated by asterisks and gaps in the alignment are indicated by hyphens. The domains of the proteins are indicated by horizontal arrows and labeled as in Fig. 1. The numbers of the first and last amino acids in each line are indicated in the left and right margins, respectively. CD8β sequences are from this study for human, from reference 12 for mouse, and from reference 14 for rat. These sequence data have been submited to the EMBL/GenBank Data Libraries under the accession number Y00805.

that is not homologous to other known proteins, except the mouse and rat homologs (Figs. 1 and 2).

Alternative Splicing Yields a Human CD8β mRNA Encoding a Longer Cytoplasmic Tail. Two of the six cDNA clones sequenced contain an additional 58 bp located at a position (between nucleotides 665 and 666) corresponding in the gene to the 3′ end of the exon encoding most of the cytoplasmic tail (Fig. 3). This insertion changes the reading frame, thereby altering the predicted cytoplasmic tail amino acid sequence and termination codon, and extending the length of the predicted protein sequence by 36 amino acids (predicted mol wt, 25,327). To determine the mechanism accounting for this additional mRNA species, we sequenced a genomic subclone containing the last exon of the gene and encoding the COOH terminus of the cytoplasmic tail and the 3′ untranslated region. This portion of the gene had not been previously sequenced. We found that the additional 58 nucleotides derive from utilization of an imperfect alternative splicing acceptor site at the 5′ end of this exon (Fig. 3). S1 nuclease studies indicate that there is a substantial amount of this alternative mRNA species present in human thymus RNA (data not shown).

Polymorphism of Human CD8β. Southern blot analysis has shown the presence of a single, nonrearranging *CD8β* gene in the human genome (data not shown). However, our cDNA clones (derived from a single individual) represent two sets of sequences with three nucleotides changes between them (Fig. 1), so these sequences must correspond to two separate alleles of CD8β. Two of these changes are in the 3′ untranslated region, while one is in the transmembrane region and results in an amino acid substitution at position 159 (Val to Ile). In comparing our cDNA sequences with both the published partial genomic sequence (17) and our additional genomic sequence (Fig. 3), we find three additional polymorphisms (nucleotides 556, 618, and 677), two of which result in amino acid substitutions in the transmembrane

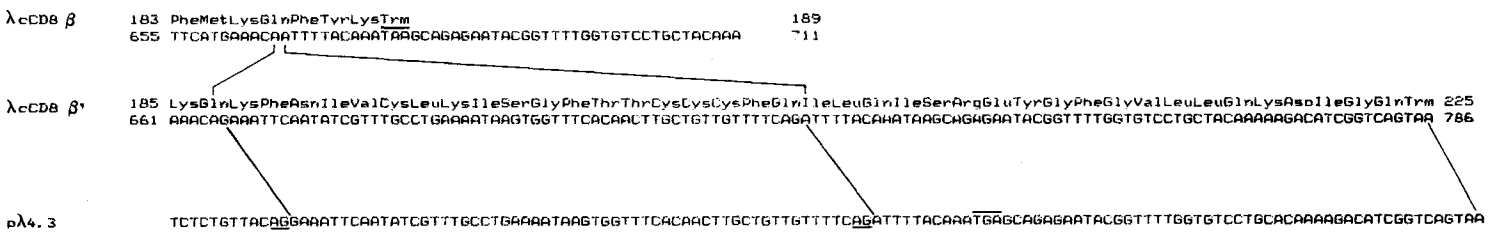

FIGURE 3. Nucleotide sequence of two CD8β clones encoding an extended cytoplasmic tail. On top (λcCD8β) is shown the 3′ end of the coding sequence (from Fig. 1) of cDNA clones encoding cytoplasmic tails similar to those of mouse and rat CD8β. In the center (λcCD8β′) is shown the 3′ coding sequence of two other cDNA clones in which 58 additional bp are present between nucleotides 665 and 666, thereby extending the open reading frame. Below (pλ4.3, reference 17) is shown the sequence of a genomic subclone indicating that the extra 58 bp derive from use of an alternative splicing acceptor site at the 5′ end of the final exon of the *CD8β* gene. The AG sequences of the two alternative acceptor sites are underlined. The normal termination codons in λcCD8β and genomic clone pλ4.3 are overlined.

region (amino acids 150 [Ile or Val] and 170 [Ile or Met]) and one (Fig. 3) that alters the termination codon (TAA or TGA).

Expression of Human CD8β mRNA. Expression of CD8β mRNA was examined by Northern blot analysis (Fig. 4). We could detect no CD8β mRNA in the T cell leukemia lines HPB-ALL (Fig. 4, lane *1*) or JM (Fig. 4, lane *2*), although both of these cell lines express CD8α mRNA and protein (Fig. 5 and data not shown). It should be noted that another line of HPB-ALL has been reported to contain CD8β mRNA (17). As has been previously observed (17), human thymus expresses a large amount of CD8β mRNA of ~1.5 kb (Fig. 4, lane *3*). Most importantly, a 1.5-kb CD8β transcript is also expressed in human PBMC (Fig. 4, lane *4*).

Expression of Human CD8β Protein on Transfected L Cells. To investigate whether human CD8β could be expressed at the protein level, a full-length cDNA was subcloned into an expression vector (pSFSVneo, reference 20) to yield pSFSVn-HCD8β. tk^- L cells were transfected with pSFSVn-HCD8β alone, or pSFSVn-HCD8β and an excess of the *CD8α* gene (pBR322/TK/CD8α, reference 12). Transfectants were selected by resistance to the antibiotic G418. We were then presented with the problem of how to detect expression of the predicted human CD8β protein, since immunoprecipitation studies of variously labeled populations of human cell lines, PBL, and thymocytes with standard anti-CD8 mAbs had failed to reveal a second subunit that could correlate with this polypeptide chain. The mAb OKT5 had been described

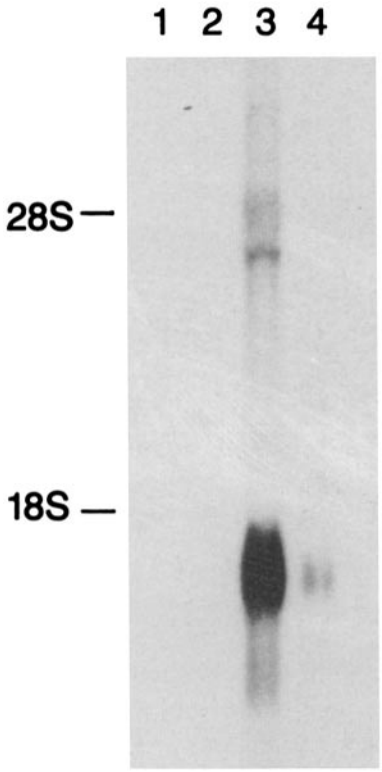

FIGURE 4. Northern blot analysis of CD8β mRNA expression. A Northern blot of total RNA from HPB-ALL (lane *1*), JM (lane *2*), human thymus (lane *3*), and human PBMC (lane *4*) was hybridized to a ^{32}P-labeled human CD8β cDNA fragment.

as binding to the same molecule as the anti-CD8 mAb OKT8, but to only a subset of OKT8$^+$ cells (24–26), so we considered the possibility that its binding might require expression of a CD8β chain. However, L cells transfected with the *CD8α* gene alone stained positively with OKT5 (data not shown). In contrast, one putative anti-human CD8 mAb among a large panel examined failed to bind to L cells transfected with the isolated *CD8α* gene (27). This mAb, 2ST8-5H7, was therefore potentially specific either for the β chain of CD8 or for a combinatorial determinant requiring heterodimer formation between CD8α and CD8β chains. As reported (27), 2ST8-5H7 did not bind to L cell transfectants expressing only CD8α (Fig. 5 *f*), although these cells did stain positively with mAb OKT8 (Fig. 5 *e*). Similarly, the HPB-ALL (Fig. 5, *a* and *b*) and JM cell lines (data not shown), which express only CD8α mRNA (see above), stained only with OKT8 and not with 2ST8-5H7. Transfectants that received only the pSFSVn-HCD8β (CD8β) construct did not stain with either OKT8 or 2ST8-5H7 (Fig. 5, *c* and *d*), although these cells expressed CD8β

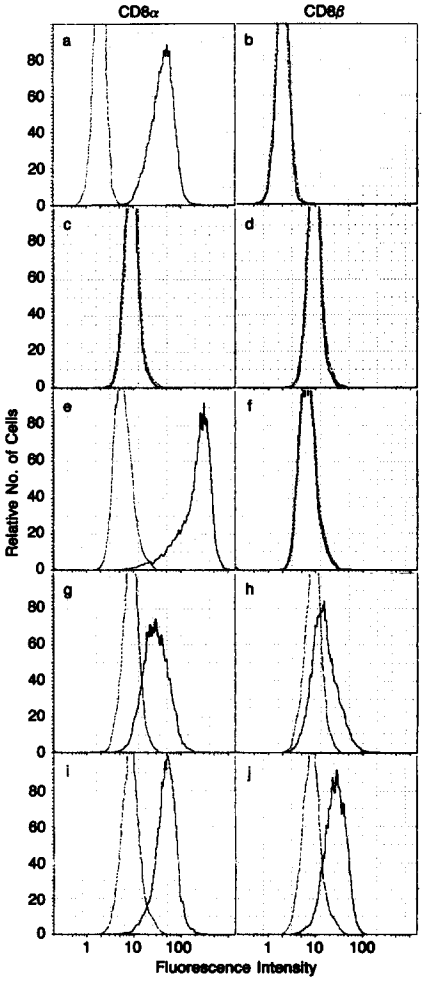

FIGURE 5. Cell surface expression of CD8α and CD8β on cell lines and transfectants. *a* and *b*, HPB-ALL; *c* and *d*, L-HCD8β-A20, a cloned line of tk$^-$ L cells transfected with the CD8β construct; *e* and *f*, L-HCD8α, tk$^-$ L cells cotransfected with the *CD8α* gene and the *tk* gene and sorted for CD8α expression after selection in HAT medium; *g* and *h*, L-HCD8αβ-P2, a twice-sorted pool of tk$^-$ L cells transfected with both CD8α and CD8β constructs; *i* and *j*, L-HCD8αβ-B4, a cloned cell line derived from L-HCD8αβ-P2. Cells were stained with either OKT8 (*a, c, e, g,* and *i*) or 2ST8-5H7 (*b, d, f, h,* and *j*), followed by fluorescein-conjugated goat anti-mouse antibody. Dotted lines represent the negative controls, which were cells stained with the second stage antibody alone. For those transfectants receiving both α and β constructs, the brightest 1% of cells staining with 2ST8-5H7 were sorted twice and then cloned using the FACS.

mRNA (not shown). In contrast, transfectants that had received constructs encoding both the α and β chains of CD8 stained positively both with OKT8 and 2ST8-5H7 (Fig. 5, *g–j*). Transfectants receiving pSFSVn-HCD8β alone were cloned randomly using the FACS, and a cloned transfectant, L-HCD8β-A20, expressing large amounts of CD8β mRNA, was derived. When the *CD8α* gene was subsequently introduced into this clone, the resultant population stained positively with both OKT8 and 2ST8-5H7 (data not shown). These data indicate that cell surface staining with mAb 2ST8-5H7 requires expression of both the α and β chains of CD8. In the mouse system we have previously shown that cell surface expression of the Ly-3 (CD8β) chain requires the presence of Ly-2 (CD8α) (12), although Ly-2 can be expressed on the cell surface as a homodimer in the absence of Ly-3 (10, 28). While it is likely that the same situation holds true for humans, we cannot rule out the possibility that our results reflect a specificity of mAb 2ST8-5H7 for a determinant unique to an α-β heterodimer rather than merely pointing to the requirement of the α chain for cell surface expression of the β chain. However, regardless of whether 2ST8-5H7 recognizes the β chain directly or only α-β heterodimers, these results demonstrate the existance of a CD8β protein that can be expressed on the cell surface of transfectants, and further imply that this chain can complex with the CD8α chain.

We also transfected tk^- L cells with a construct encoding the alternative CD8β chain that has a longer cytoplasmic tail sequence (Fig. 1 *b*), either alone or together with the *CD8α* gene. The resulting transfectants did not stain positively with 2ST8-5H7 despite the presence of CD8β mRNA in the cells (data not shown), suggesting that the predicted alternative polypeptide chain cannot be expressed on the cell surface.

Expression of Human CD8β on the Surface Peripheral Blood T Cells. With the knowledge that a CD8β protein could be expressed, we examined the presence of this protein chain in normal human peripheral blood cells to see whether the biologically relevant form of CD8 might be a heterodimer in humans as it is in mice and rats. We found that the determinant defined by 2ST8-5H7 is indeed expressed on PBMC, and that it is present only on $OKT8^+$ ($CD8\alpha^+$) cells (Fig. 6). Approximately 17% of PBMC stained positively with OKT8 (Fig. 6 *a*), 16% stained positively with 2ST8-5H7 (Fig. 6 *b*), and 16% with both mAbs (Fig. 6 *c*). The percentage of cells staining positively with OKT8 was consistently slightly higher than that of cells staining positively with either 2ST8-5H7 alone or both mAbs. The population of cells that are $CD8\alpha^+$ and $CD8\beta^-$ comprises no more than 1% of PBMC. A small percentage of the double positives seem to show a slightly reduced level of staining with 2ST8-5H7, resulting in significant "shoulder" in the positive population (Fig. 6 *c*). This may reflect a difference in subunit composition of heteromultimers, or it may be an indication that homodimers of the α chain exist as well. These data do not allow us to conclude what proportion of CD8 molecules are α homodimers (or homomultimers) vs. α-β heterocomplexes.

Discussion

Biochemical studies of the subunit composition of CD8 in mice, rats, and humans indicated some surprising interspecies differences considering the similarities in both function and cellular distribution of CD8 among these species. Mouse CD8 was found to consist of two forms of disulfide-linked heterodimers (αβ and α′β), while one form of disulfide-linked heterodimer (αβ) was identified in rat (8, 9, 13, 14).

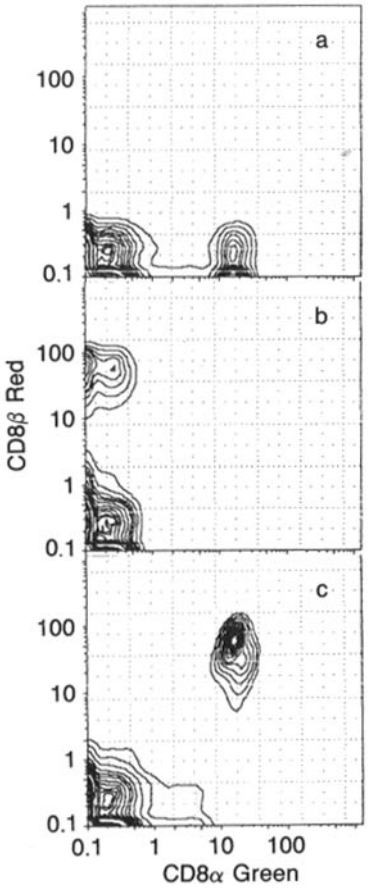

FIGURE 6. Cell surface expression of CD8α and CD8β on normal human PBMC. Normal human PBMC were stained with (*a*) fluorescein-conjugated OKT8 (Ortho Diagnostic Systems, Inc., Westwood, MA); (*b*) 2ST8-5H7 and Texas Red–conjugated goat anti-mouse antibody (Tago Inc., Burlingame, CA); (*c*) antibodies used in both *a* and *b*. Stainings were performed sequentially in a buffer of 1 × PBS, 0.1% BSA, 0.02% sodium azide, and 10% heat-inactivated rabbit serum. A washing step with 25% normal mouse serum was also included to block crossreactivity. Data are presented as 5% probability contour plots. Large cells such as macrophages were eliminated from the analysis by placing gates on the forward and obtuse scatter.

In contrast, human CD8 was believed to consist of homodimers and homomultimers of a single (15, 16) chain equivalent to mouse CD8α (10, 11). In the thymus, but not in the periphery, the higher multimers of human CD8 were found to be disulfide linked to a 46-kD CD1 glycoprotein (16, 29, 30). CD1 is a class of molecules related to class I MHC proteins, although they are encoded on a different chromosome (31). While CD1 is typically associated noncovalently with β_2-microglobulin (31, 32), no β_2-microglobulin has been found associated with the CD1 that is linked to CD8 in human thymus (29, 30). Mouse and rat CD8 have not been described as being associated with a CD1-equivalent protein in thymus.

Molecular biological studies have now resolved many of the discrepancies in CD8 structure in mice, rats, and humans. The α and α′ chains of mouse CD8 (Ly-2α and Ly-2α′) have been shown to be products of alternatively spliced mRNA species derived from a single *CD8α* gene (10, 28). The exclusion of sequence from exon IV of this gene results in a frame-shift mutation, early termination, and production of α′ protein. The α′ chain differs from the α chain (which contains exon IV sequence) only in the cytoplasmic tail, which is four amino acids in length instead of 29. A human or rat equivalent of the α′ chain has not been seen at the protein level, and

we have not detected an equivalent alternatively spliced form of human CD8 mRNA (unpublished results). However, it is unlikely that the absence of a human or rat CD8α′ chain results in any functional difference in mature T cells. Several laboratories have found that very little α′ chain is expressed on the surface of mouse peripheral T cells, despite the fact that thymocytes express close to equal amounts of cell surface α and α′ chains (9, 28, 33). We have recently demonstrated that this is a result of a post-translational regulatory mechanism that blocks surface expression of the α′ chain in peripheral T cells and the most mature subset of $CD8^+$, $CD4^-$ thymocytes (33). While it is possible that the α′ chain plays a role during thymocyte development, it is clear that it is not an important molecule on mature mouse T cells. Similarly, the absence of association of mouse and rat CD8 with CD1 on thymocytes is not likely to be functionally significant, since the dimeric form of human thymocyte CD8 also lacks CD1, and since CD1 is not expressed on peripheral T cells.

The final discrepancy between human and rodent CD8 is addressed by the data presented here. No CD8β (Ly-3 equivalent) chain has been identified as being associated with CD8α in biochemical studies of human CD8. However, we have recently demonstrated that human CD8α, like its counterpart, can be used to rescue cell surface expression of mouse CD8β (12). These findings suggested that human CD8α is indeed capable of associating with a β chain. We have now shown that mRNA encoding a CD8β chain is expressed in both human thymus and PBMC, and the sequences of cDNA clones encoding this chain contain an open reading frame. We further found that a CD8β chain could be expressed on the surface of L cells transfected with a human CD8β cDNA clone in an expression vector. Binding of the mAb 2ST8-5H7 to transfectants required the presence of both α and β chains of CD8, either because the mAb binds to a conformational determinant that requires the presence of both chains, because CD8α is required for surface expression of CD8β (as in the mouse system), or both. A second form of human CD8β cDNA could be shown to be the product of an alternatively spliced form of CD8β mRNA and to encode a protein with a longer cytoplasmic tail. However, a product of this form of cDNA was not detected on the cell surface of transfected L cells, suggesting that the encoded protein either fails to bind to CD8α or results in a complex that is excluded from the cell surface. Finally, we demonstrated that human CD8β is expressed on the surface of almost all PBMC that express CD8α. The small population of cells (1%) that were positive for CD8α and negative for CD8β could represent a different subset from the normal $CD8^+$ T cells bearing αβ TCRs; eg., they could represent NK cells or another type of T cell. We conclude that the structure of CD8 on peripheral blood T cells is essentially the same in mouse, rat, and man.

It is unclear why the presence of the CD8β chain has not been demonstrated previously. Possibilities include inability to radiolabel well and/or comigration with the α chain. If the latter is true, the CD8β protein must contain a large amount of *O*-linked glycosylation, since the predicted molecular mass based on amino acid sequence is 21.4 kD and the sequence only predicts one site of *N*-linked glycosylation. Immunoprecipitation of CD8 has in some cases yielded a broad band or two closely spaced bands on one dimensional SDS-PAGE (15, 16, 34, 35), but protein sequencing of the two bands of the CD8 doublet purified by Snow et al. (35) yielded a single polypeptide sequence. It is possible that this resulted from a blocked NH_2 terminus of a closely migrating CD8β chain, however, tryptic peptide mapping of the two

species were indistinguishable (35). Unfortunately, mAb 2ST8-5H7 has been found not to immunoprecipitate any protein molecules from the surface of human PBL (27). However, the availability of cDNA clones encoding CD8β should allow the generation of new mAbs specific for this chain, and such mAbs could then be used to determine the reason that human CD8β has thus far not been identified as a protein band on a gel.

Gene transfer experiments with the mouse *CD8α* gene have demonstrated that homodimers of α and/or α′ chains can perform at least some of the functions attributed to CD8, despite the fact that the physiological form of this protein is a heterodimer containing a β chain (5–7). mAbs specific for the mouse β chain (as well as those specific for the α chain) have been shown to inhibit cytotoxicity by mouse $CD8^+$ T cells (8, 36). It will be of great interest to determine in both mice and humans whether the presence of the β chain modifies or adds additional functional properties to the CD8 molecule.

Summary

Human CD8 has been thought to consist of disulfide-linked homodimers and homomultimers of a single polypeptide chain homologous to mouse and rat CD8α. In contrast, mouse and rat CD8 are composed of disulfide-linked heterodimers of α and β chains. We have now isolated and sequenced cDNA clones encoding a human homologue of mouse and rat CD8β. One such clone was inserted into an expression vector and its encoded product was shown to be expressed on the cell surface after cotransfection into L cells with the human *CD8α* gene. A second form of human CD8β cDNA encoding a protein with an altered cytoplasmic tail was similarly transfected, but its product could not be demonstrated on the cell surface. CD8β was further shown to be expressed on the surface of almost all $CD8^+$ human peripheral blood T cells. These data provide the first evidence that human CD8 is a heterodimeric protein.

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