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Immunology

A secreted form of the human lymphocyte cell surface molecule CD8 arises from alternative splicing

(lymphocyte differentiation antigen/gene evolution/immunoglobulin superfamily)

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ABSTRACT **The human lymphocyte differentiation antigen CD8 is encoded by a single gene that gives rise to a 33- to 34-kDa glycoprotein expressed on the cell surface as a dimer and in higher molecular mass forms. We demonstrate that the mRNA is alternatively spliced so that an exon encoding a transmembrane domain is deleted. This gives rise to a 30-kDa molecule that is secreted and exists primarily as a monomer. mRNA corresponding to both forms is present in peripheral blood lymphocytes, Con A-activated peripheral blood lymphocytes, and three CD8$^+$ T-cell lines, with the membrane form being the major species. However, differences in the ratio of mRNA for membrane CD8 and secreted CD8 exist. In addition, the splicing pattern we observe differs from the pattern found for the mouse CD8 gene. This mRNA is also alternatively spliced, but an exon encoding a cytoplasmic region is deleted, giving rise to a cell surface molecule that differs in its cytoplasmic tail from the protein encoded by the longer mRNA. Neither protein is secreted. This is one of the first examples of a different splicing pattern between two homologous mouse and human genes giving rise to very different proteins. This represents one mechanism of generating diversity during speciation.**

CD8 is an important molecule expressed on the surface of a subset of T cells, generally the cytotoxic/suppressor cells, as well as on some natural killer cells (1). Functionally, CD8 appears to interact with major histocompatibility complex (MHC) class I molecules either on the same cell (2) or on target cells where it facilitates cell–cell interactions of T cells recognizing foreign antigen in association with MHC class I molecules (3). In addition, it has been reported that CD8 can associate with the T-cell antigen receptor (4, 5) and that phosphorylation of CD8 occurs upon T-cell triggering (6, 7). In humans the CD8 molecule is a 33- to 34-kDa glycoprotein found as a homodimer and in larger forms (tetramers) on peripheral blood lymphocytes (8). However, on thymocytes, some larger forms of CD8 contain CD1a, a 49-kDa glycoprotein (9–11). Mouse CD8 (Lyt-2) from the thymus is composed of two polypeptides of 38 kDa (Lyt-2α) and of 34 kDa (Lyt-2α'), usually disulfide-linked to a 30-kDa Lyt-3 polypeptide encoded by a closely linked gene (12). The 38-kDa and 34-kDa polypeptides arise by alternative splicing of mRNA transcribed from a single mouse CD8 gene (13).

Levy and coworkers (14) first reported that soluble human CD8 could be detected in the supernatant of transformed T-cell lines. A small amount of soluble CD8 was found in the serum of normal individuals. However, in some patients who were positive for T-cell acute lymphocytic leukemia, high amounts of CD8 were detectable in the serum. It was postulated that the soluble form, a 27-kDa monomer by SDS/PAGE under both reducing and nonreducing conditions, was released from the surface by a specific proteolytic cleavage, since radioactive CD8 was found in the supernatant after surface radioiodination of the T-cell line HPB-ALL (15).

In our studies we analyzed two types of CD8 cDNA clones from an Okayama–Berg cDNA direct expression library, constructed from mRNA of a mouse L-cell line transfected with human DNA and selected for expression of CD8 (16, 17). There was no indication of two forms of CD8 mRNA by Northern blot analysis, in which a single 2.5-kilobase (kb) band was detectable (18); yet 2 out of 10 apparently full-length clones picked from this library lacked an *Eco*RV restriction enzyme site that was within the exon encoding the transmembrane domain, whereas other restriction sites were identical.

We have characterized both types of cDNAs and found that the predominant clone encodes the typical membrane form of CD8 whereas the other cDNA encodes a soluble form of human CD8.§ Both forms are present in activated and unactivated peripheral blood lymphocytes as well as in three CD8$^+$ T-cell lines. Therefore, an active secretory mechanism exists for producing soluble CD8.

MATERIALS AND METHODS

Cells and Cell Culture. Epstein–Barr virus (EBV)-transformed, hypoxanthine phosphoribosyltransferase (HPRT)-deficient human lymphoblastoid UC729-6 (UC) cells (19) were obtained from R. Levy (Stanford) and maintained in RPMI-1640 medium containing penicillin, streptomycin, and 10% fetal bovine serum (FBS) in 5% CO_2/95% air at 37°C. The five T-cell lines DND, Jurkat, JM, MOLT-4, and HPB-ALL were maintained similarly. The HPB-ALL line was a kind gift of A. Weiss (University of California, San Francisco). The other cell lines were obtained from L. Herzenberg's laboratory, which received them from J. Minowada (Roswell Park Memorial Institute, Buffalo, NY). The peripheral blood lymphocytes were obtained from the Yale Blood Bank and isolated on Ficoll/Hypaque density gradients. After incubation on plastic for 30 min at 37°C to remove most monocytes, the cells were cultured at 5×10^5 per ml in RPMI-1640 with Con A (10 μg/ml, Sigma) and 10% FBS at 37°C for 4 days.

Transfection of Human Lymphoblastoid Cells. The procedure was as follows (17, 20). UC cells (5×10^6) in logarithmic growth phase were pelleted, washed with Dulbecco's phosphate-buffered saline, and then suspended in 1.0 ml of HBS (20 mM Hepes/137 mM NaCl/5 mM KCl/0.7 mM Na_2HPO_4/6 mM dextrose, pH 7.05) containing 10 μg of EBO-pcD DNA and 300 μg of sheared salmon sperm DNA. Cells and DNA in HBS were kept at room temperature for 10–15 min and then electroporated with a capacitor discharge of 250 V and 960 μF

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Abbreviations: MHC, major histocompatibility complex; EBV, Epstein–Barr virus; FACS, fluorescence-activated cell sorter; V, variable.

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§The sequences reported in this paper are being deposited in the EMBL/GenBank data base (accession no. J04165).

(Bio-Rad). The transfected cells were kept at room temperature for an additional 10–15 min and then diluted into RPMI-1640 with 15% FBS to 5×10^5 cells per ml. Cell survival the next day was 10–30%. Selection for hygromycin B-resistant cells was begun 48 hr posttransfection, with hygromycin B (Calbiochem) at 100 μg/ml for 2 days and at 200 μg/ml thereafter. The hygromycin B was diluted in phosphate-buffered saline with Hepes (pH 7.4) and stored at −20°C.

Fluorescence-Activated Cell Sorter (FACS) Analysis. Cells were washed with phosphate-buffered saline and resuspended in staining medium [biotin-free RPMI-1640 (Irvine Scientific)/10 mM Hepes, pH 7.4/0.1% NaN_3/1% FBS). For analysis 0.5×10^6 cells were incubated with a saturating amount of anti-CD8 antibody (anti-Leu2a; Becton Dickinson Monoclonal Center) for 25 min at 0°C in 50 μl of staining medium in a round-bottomed well of a polypropylene microtiter plate. Cells were washed once with 175 μl of staining medium and incubated with fluorescein-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL) for 20 min. Propidium iodide (Calbiochem) was added to a final concentration of 1 μM. After 5 min, the cells were centrifuged and washed with staining medium. Cells were analyzed with a FACStar flow cytometer (Becton Dickinson FACS Systems).

Detection of Soluble CD8. Cells were seeded at 2×10^5 per ml, and after 72 hr supernatant was collected. The cells were pelleted ($200 \times g$, 10 min), and then the supernatant was centrifuged in an Eppendorf centrifuge ($15{,}000 \times g$, 10 min) to remove any remaining cells. An ELISA test kit (T Cell Sciences) was used to measure the amount of soluble CD8.

Cell Labeling, Immunoprecipitation, and Gel Electrophoresis. Cells were surface-labeled with ^{125}I by using lactoperoxidase as described (9). Cells were lysed with buffer containing 0.5% Nonidet P-40, and immunoprecipitates were obtained by adding 20 μg of anti-monoclonal antibody CD8 G10-1 (21) followed by 50 μl of packed protein A-Sepharose (Pharmacia). For biosynthetic labeling, cells (2×10^7 in 10 ml) were incubated for 3 hr at 37°C in methionine-free RPMI-1640 with dialyzed FBS and [^{35}S]methionine (100 μCi/ml; 1 μCi = 37 kBq). Supernatants were immunoprecipitated with G10-1 as above. Immunoprecipitates were analyzed by SDS/PAGE (22) in 10% gels. Molecular weight markers (Pharmacia) run in each gel were visualized by staining with Coomassie blue.

Sequence Analysis. Fragments for sequencing were subcloned into the pGEM-3z vector (Promega) and sequenced by the Sanger dideoxy method using the Sequenase kit (United States Biochemical) with [α-[^{35}S]thio]dATP.

RNase Protection Assay. RNA was extracted from cells by the guanidinium thiocyanate method (23) and subsequently separated in a 5.7 M CsCl gradient. The probe was an *Eco*RV–*Xho* II fragment from the cDNA encoding the membrane form of CD8, subcloned into the pGEM-3z vector. The plasmid was linearized with the restriction enzyme *Bgl* II and extensively extracted with phenol/chloroform, 1:1 (vol/vol). The reaction was carried out as described by Melton *et al.* (24) by using a Promega kit with 1.0 μg of DNA, two labeled nucleotides ([α-^{32}P]CTP and [α-^{32}P]UTP), and incubation at 40°C for 60 min. Phage SP6 RNA polymerase was used to produce an antisense strand. After addition of DNase and incubation for 15 min at 37°C, 3 μl of 0.5 M EDTA was added and the sample was extracted once with phenol/chloroform. The sample was loaded onto a Sephadex G-50 spin column and the labeled probe was recovered by centrifuging the column. After precipitation with tRNA as carrier, the probe was resuspended in 5 mM Tris (pH 7.5) and dried down with 50 μg of mRNA. Thirty microliters of hybridization buffer (80% deionized formamide/40 mM Pipes, pH 6.7/400 mM NaCl/1 mM EDTA) was added and, after mixing, the sample was heated to 90°C for 5 min and incubated at 45°C overnight. At room temperature, 300 μl of RNase solution (0.3 M NaCl/10 mM Tris, pH 7.5/5 mM EDTA with RNase A at 40 μg/ml and RNase T_1 at 2 μg/ml) was added. After 60 min at 30°C, 20 μl of 10% SDS and 2.0 μl of proteinase K (25 mg/ml) was added. After 15 min at 37°C, extraction with phenol/chloroform was performed and the nucleic acids were ethanol-precipitated along with carrier tRNA. The samples were electrophoresed in an 8% polyacrylamide gel with 8 M urea in 2× TBE buffer (1× TBE is 89 mM Tris/89 mM boric acid/2 mM EDTA). The running buffer was 2× TBE. Kodak XAR film was used for autoradiography.

RESULTS

Expression of Alternative Forms of cDNA. We transfected the two types of cDNA clones into a human B-lymphoblastoid cell line, UC, by electroporation and asked whether a membrane or secretory form of CD8 could be detected. The Okayama–Berg plasmid containing each CD8 cDNA was first modified by addition of a DNA fragment containing the EBV origin for plasmid replication (oriP), EBV nuclear antigen (EBNA-1) gene, and the hygromycin phosphotransferase gene as selectable marker (17) (Fig. 1). Plasmids containing the EBV oriP sequence are able to replicate autonomously in a B-lymphoblastoid line (25) and allow for high transfection frequencies. Two days after electroporation, the cells were grown in medium containing hygromycin B to allow selection of stable transfectants. About 20% of the cells were viable after electroporation, and about 10% of these cells were hygromycin B-resistant.

We analyzed stable transfectants for cell surface expression of human CD8 as well as the presence of CD8 in the supernatant. UC cells transfected with plasmids whose cDNA contained the *Eco*RV restriction site (UC-mCD8 cells) expressed CD8 on the surface as assayed by FACS analysis of cells with anti-CD8 antibody. However, cells transfected with plasmids whose cDNA lacked the *Eco*RV restriction site (UC-sCD8 cells) failed to express detectable CD8 on the surface (Fig. 2). In contrast, UC-sCD8 transfectants had

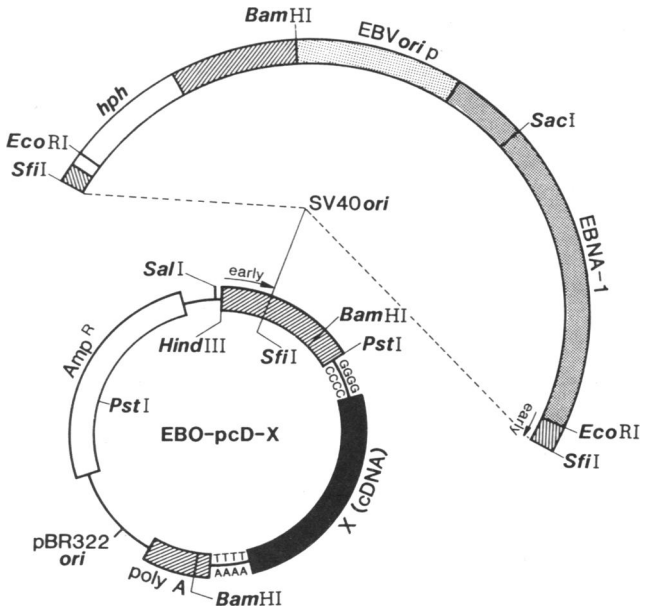

FIG. 1. Structure and component parts of the EBO-pcD-cDNA plasmid (17). Okayama and Berg (36) described the pcD-cDNA plasmid, in which a cDNA is transcribed under control of the simian virus 40 early promoter. Into the *Sfi* I site of the plasmid was inserted a fragment containing the hygromycin phosphotransferase gene (*hph*) for selection in mammalian cells, the EBV origin for plasmid replication (oriP), and the EBNA-1 gene, which encodes a transactivating factor for oriP.

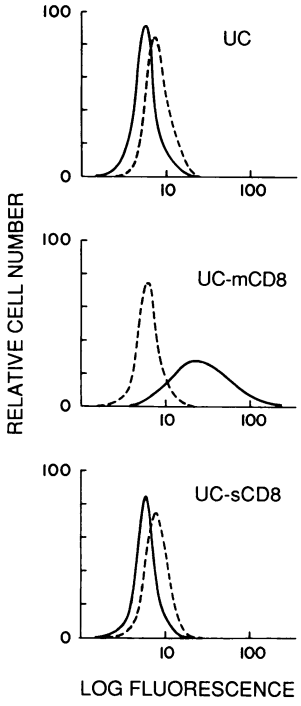

FIG. 2. FACS fluorescence intensity histograms of UC lymphoblastoid cells and UC cells transfected with plasmids containing cDNA for the membrane (UC-mCD8) or secreted (UC-sCD8) form of CD8. Cells were stained by incubation with anti-CD8 monoclonal antibody (anti-Leu2a) as first reagent followed by fluorescein-labeled goat anti-mouse IgG as second reagent (—) or by incubation with second reagent alone (---). UC-mCD8 transfectants expressed CD8 on the cell surface, whereas UC-sCD8 transfectants and untransfected UC cells had no detectable surface CD8.

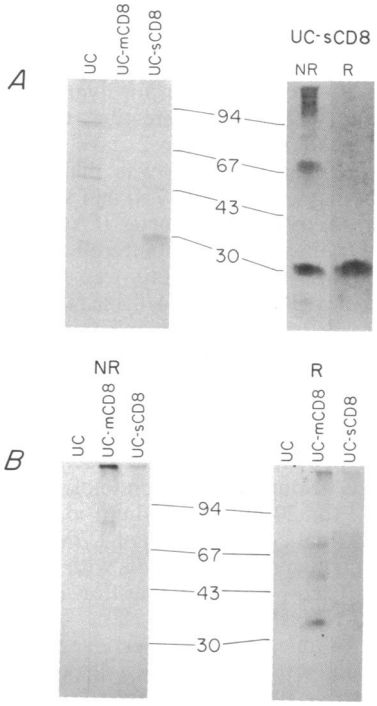

FIG. 3. Characterization of CD8 produced by transfectants of the UC cell line. (*A*) The parental UC cell line and the UC-mCD8 and UC-sCD8 transfected cells were labeled with [^{35}S]methionine for 3 hr at 37°C and the supernatants were then analyzed by immunoprecipitation with anti-CD8 monoclonal antibody G10-1. On the left, the immunoprecipitates were analyzed by SDS/PAGE after reduction of disulfide bonds. On the right, an immunoprecipitate from the supernatant of UC-sCD8 was analyzed without reduction (NR) or with reduction (R) as indicated. (*B*) The UC cell line and CD8 transfectants were surface-labeled with ^{125}I, and cell lysates were immunoprecipitated with G10-1. The immunoprecipitates were analyzed without reduction (NR) or with reduction (R) as indicated. Positions of molecular size markers (in kilodaltons) are shown.

about 7 times as much soluble CD8 in the supernatant as compared with UC-mCD8 transfectants by ELISA (Table 1). Soluble CD8 was detected in the supernatants from both sets of transfectants but not in the supernatant from the untransfected recipient UC cell line.

Biochemical Characterization of Soluble CD8. Cells were biosynthetically labeled with [^{35}S]methionine for 3 hr at 37°C and the supernatants were immunoprecipitated with anti-CD8 antibody. A 30-kDa molecule was detected only in supernatant from the UC-sCD8 cells, which lacked CD8 surface expression. When the CD8 precipitate from the UC-sCD8 supernatant was analyzed in reducing and nonreducing SDS/polyacrylamide gels, the 30-kDa molecule was the predominant form even without reduction, and only small amounts of higher molecular forms were present (Fig. 3*A*). In contrast to the UC-sCD8 cells, a typical 33- to 34-kDa monomer of CD8 was detected on the UC-mCD8 transfectants when analyzed under reducing conditions, with all of the CD8 existing as higher molecular forms when analyzed without reduction of disulfide bonds (Fig. 3*B*).

Table 1. CD8 in supernatants from transfectants and CD8$^+$ cell lines

Cells	CD8, units/ml
UC	35
UC-mCD8	
Transfection 1	150
Transfection 2	175
UC-sCD8	
Transfection 1	1200
Transfection 2	1100
JM	260
MOLT-4	400
HPB-ALL	320

CD8 was detected by ELISA. UC-mCD8 and UC-sCD8 cells from two separate transfections were analyzed independently.

Sequence Differences Between cDNAs Encoding Soluble CD8 and Membrane CD8. We performed restriction enzyme analysis and sequence analysis to compare the two forms of the cDNAs. The coding portions of the two forms were identical until 11 amino acids before the transmembrane domain. The cDNA encoding the secreted protein lacks 111 base pairs (bp) that correspond exactly to a 111-bp exon in the genomic clone. This results in the removal of 11 amino acids in the hinge-like region, the entire transmembrane domain, and several amino acids from the beginning of the cytoplasmic domain (Fig. 4). There is no change in reading frame, so that the rest of the cytoplasmic domain is identical. This results in a loss of 38 amino acids and the addition of a glycine encoded by bases at the splice junction for a total loss of 37 amino acids.

Interestingly, the cysteine just before the transmembrane domain is lost as well as two cysteines in the transmembrane domain. There are nine cysteines in CD8: three in the region that resembles the immunoglobulin variable region (V-like region), two in the hinge-like region, two in the transmembrane region, and two in the cytoplasmic domain (26, 27). To form the high molecular weight complexes that are composed of more than two CD8 monomers, at least two of these cysteines are involved in disulfide linkage to other monomers. Two of the three cysteines in the V-like domain are probably disulfide-bonded to each other to form the immunoglobulin globular structure, leaving three remaining cysteines on the outside of the cell for potential pairing. Because

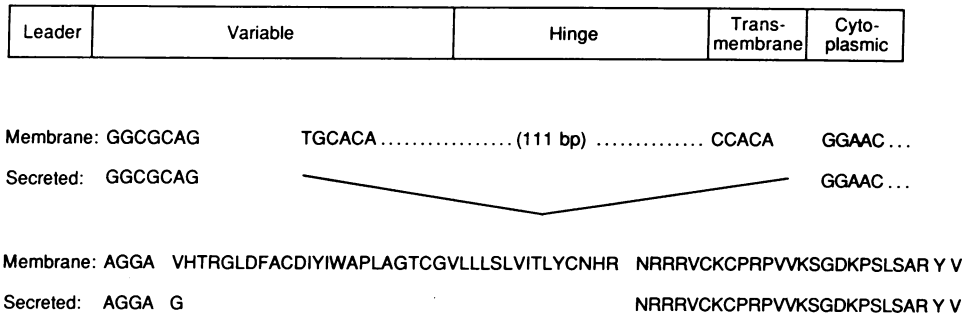

FIG. 4. Comparison of the membrane and secreted forms of CD8. (*Top*) Schematic representation of the membrane-form mRNA. (*Middle*) Sequences encoding the membrane and secreted forms, indicating the loss of 111 bp from the secreted form. (*Bottom*) Carboxyl-terminal amino acid sequences (one-letter symbols) of the two forms.

secreted CD8 has reduced ability to pair with other monomers, it is likely that the deleted cysteine near the transmembrane domain is important in intermolecular disulfide linkage.

RNase Protection Analysis. To assess the physiologic relevance of the two forms of cDNA corresponding to membrane or secreted CD8, we performed RNase protection assays with mRNA from 4-day Con A-activated and unactivated peripheral blood lymphocytes as well as several T-cell lines. The *Eco*RV–*Xho* II probe was 240 bp long and spanned the transmembrane and cytoplasmic domains (Fig. 5 *Upper*). mRNA corresponding to the membrane form or the secreted form of CD8 would give protected fragments of 197 bp or 118 bp respectively. mRNAs for both membrane and secreted forms of CD8 were present in both resting and activated T cells as well as in the $CD8^+$ T-cell lines HPB-ALL, JM, and MOLT-4 but not in two $CD8^-$ cell lines, Jurkat and DND (Fig. 5 *Lower*). The ratio of membrane CD8 mRNA to secretory CD8 mRNA was calculated from densitometry of the autoradiograms. In all cases the membrane form was predominant, although the ratio was variable. The values were 26.5 (HPB-ALL), 6.8 (JM), 6.8 (MOLT-4), 9 (activated, donor A), 14.7 (activated, donor B), and 2.2 (unactivated, donor A). The extra bands near the 197- and 118-bp bands are presumably a result of extra RNase digestion at the ends of the RNA·RNA hybrids during RNase A treatment.

DISCUSSION

The CD8 gene encodes two forms of the protein, a membrane form and a soluble form. We have shown that these two forms arise from alternatively spliced mRNAs in which the transmembrane domain exon is deleted from the mRNA encoding the soluble form. The soluble form is 30 kDa and has significantly reduced ability to form dimers and larger complexes. mRNAs for both forms are found in both activated and unactivated peripheral blood lymphocytes, with the mRNA for the membrane form being predominant in both.

Although both the mouse and human CD8 genes are alternatively spliced, the splicing pattern is quite different. The mouse CD8 gene is organized into five exons: a fused leader and V-like exon, a hinge exon, a transmembrane exon, and two intracytoplasmic exons (28). The first cytoplasmic exon is spliced out so that both Lyt-2 proteins contain transmembrane domains and are expressed on the cell surface (12, 13). In contrast, the reading frame changes so that the cytoplasmic tails differ in length and sequence.

In the human CD8 gene the alternative form results from splicing of the exon corresponding to the transmembrane domain. Both mouse and human genes have separate transmembrane exons that are the same length (111 bp) and very homologous (79% amino acid sequence identity). In addition, the splice donor and acceptor for splicing of the hinge-like exon to the transmembrane exon are identical (AG–TG), and the splice donor and acceptor for splicing of the transmembrane exon to the cytoplasmic exon are similar (human, CA–GG; mouse, CA–GC). The exon structure for the rest of the human gene is like that of the mouse gene except that the leader and V-like region are encoded by separate exons (H. Nakauchi, personal communication).

Whether the difference in splicing results from differences in the sequence or from differences in splicing mechanisms between species is unknown. However, since normal splicing patterns are observed when genomic Lyt-2 or genomic CD8

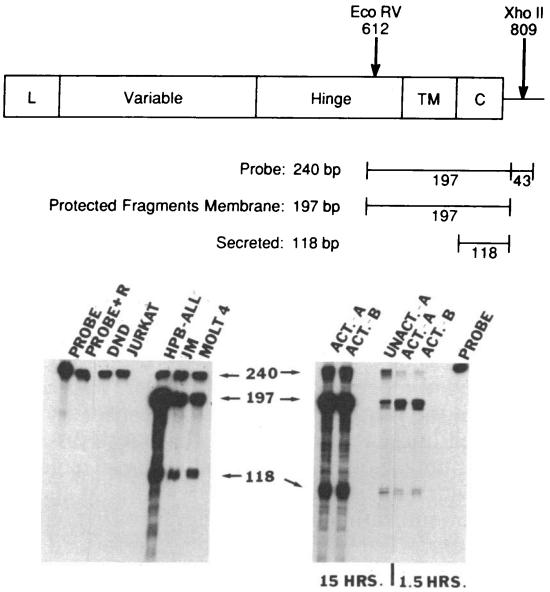

FIG. 5. RNase protection assay. (*Upper*) A pGEM-3z vector containing the *Eco*RV–*Xho* II fragment from membrane-CD8 cDNA was linearized with *Bgl* II, and RNA was transcribed from the SP6 promoter. This resulted in a 240-bp probe that contained 43 bp of linker sequence and 197 bp of cDNA sequence. mRNA encoding the membrane form of CD8 would give rise to a protected fragment of 197 bp, whereas the mRNA for the secreted form would give rise to a protected fragment of 118 bp. (*Lower*) mRNA from three $CD8^+$ T-cell lines (HPB-ALL, JM, and MOLT-4) and two $CD8^-$ T-cell lines (DND and Jurkat) was analyzed for the presence of the protected fragment corresponding to membrane CD8 (197 bp) and to secreted CD8 (118 bp). A band corresponding to partially digested probe alone (240 bp) was present in the DND and Jurkat lines, but not the two bands (197 and 118 bp) corresponding to CD8 present in the three $CD8^+$ cell lines. The two CD8 bands were also present with mRNA with unactivated peripheral blood lymphocytes of donor A and from Con A-activated lymphocytes of donor A and donor B. The film was exposed for two different times (15 hr and 1.5 hr) because the amount of CD8 mRNA in the activated cells was much greater. Lanes labeled PROBE had 10^4 cpm of labeled probe. Lanes labeled PROBE + R indicate samples containing tRNA (50 μg) and the same amount of probe used in other reactions (2×10^6 cpm), digested with RNase A and RNase T_1 as described.

is transfected into mouse L cells (12), it is likely that the splicing differences between mouse and human are a function of specific splice sequences in the genomic DNA. Nonetheless, it is most interesting that this is one of the first examples of two homologous genes that are spliced differently in each species, leading to two very different types of proteins. This provides a mechanism whereby evolutionary diversity can be generated by using the same gene.

Soluble CD8 of ≈27 kDa was described by Fujimoto *et al.* (14) and was thought to be derived from proteolytic cleavage of cell surface CD8 because radiolabeled CD8 appeared in the supernatant after surface labeling of cells. However, it is possible that this resulted from the presence of a secreted form of CD8. If this molecule came to the cell surface complexed to membrane-bound CD8 or if it could rapidly associate and dissociate with the cell surface, then this could produce a similar result. This possibility is supported by our observation that secreted CD8 exists predominantly as a monomer but can also associate into larger forms. Serum β_2-microglobulin can associate with MHC class I molecules on the surface of cultured cells (29). It is not clear, however, whether the 27-kDa CD8 described by Fujimoto *et al.* is the same as the 30-kDa secreted CD8 that we describe. This difference in size could be due to differences in CD8 glycosylation in the lymphoblastoid cell line transfectant compared to the leukemic T-cell line HPB-ALL studied by Fujimoto *et al.* Because the UC-mCD8 transfectants showed some soluble CD8 in the supernatant, it might be argued that this resulted from proteolysis. We have not ruled out the possibility that some membrane-associated CD8 was released by cells that died during the 72 hr of *in vitro* culture. Therefore, we cannot exclude proteolytic cleavage as an alternative source of soluble CD8, but we believe that there are plausible reasons why the soluble CD8 observed by Fujimoto *et al.* may have arisen by active cell secretion.

Differences in expression of the two forms of CD8 could provide clues as to the physiologic role of the soluble form. In mouse, differences in the percentage of the two forms were noted whereby Lyt-2α mRNA made up 45% in thymocytes, 20% in lymph nodes, and 15% in a cytotoxic T-cell line (12). In the limited number of samples examined, we found some variability in the ratio of the two forms, especially between human T-cell lines. It is interesting that the HPB-ALL line expresses a human homolog of the mouse Lyt-3 molecule that associates with mouse CD8 (30), whereas the JM and MOLT-4 lines do not, indicating possible differences in their stage of differentiation. More work needs to be performed to determine the ratio in different cell types expressing CD8 (i.e., cytotoxic, suppressor, or natural killer cells) or in cells at different stages of differentiation. In addition, the ratio may vary depending upon the pathway of activation.

There are a number of possible functions of secreted CD8 in effector mechanisms or immune regulation. The secreted CD8 may block interactions between cytotoxic or suppressor T cells or $CD8^+$ natural killer cells and their targets. Alternatively, it could bind directly to the effector or target cell via a MHC molecule or by association with the membrane form of CD8, which normally complexes with other CD8 molecules.

There are now many examples of lymphocyte membrane molecules that can exist in a soluble form. Some are released by proteolytic digestion, as may be the case for the soluble interleukin 2 receptor (31), and some are released by cleavage of a phosphatidylinositol linkage, as occurs for the Fc receptor type III (32). Another mechanism is alternative splicing of the mRNA to give rise to a membrane and a secreted form. Examples of this include HLA (33), immunoglobulin (34), and possibly tumor necrosis factor, for which both membrane and secreted forms exist (35). The variety of means that lymphocytes use to release molecules that can interact with other cells reflects the complexity and diversity of lymphocyte communications.

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