Human Myeloid Plasma Membrane Glycoprotein CD13 (gp150) Is Identical to Aminopeptidase N

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Abstract

To determine the primary structure of CD13, a 150-kD cell surface glycoprotein originally identified on subsets of normal and malignant human myeloid cells, we isolated the complete sequences encoding the polypeptide in overlapping complementary DNA (cDNA) clones. The authenticity of our cDNA clones was demonstrated by the ability of the coding sequences, subcloned in a retroviral expression vector, to mediate expression of bona fide CD13 molecules at the surface of transfected mouse fibroblasts. The nucleotide sequence predicts a 967 amino acid integral membrane protein with a single, 24 amino acid hydrophobic segment near the amino terminus. Amino-terminal protein sequence analysis of CD13 molecules indicated that the hydrophobic segment is not cleaved, but rather serves as both a signal for membrane insertion and as a stable membrane-spanning segment. The remainder of the molecule consists of a large extracellular carboxyterminal domain, which contains a pentapeptide consensus sequence characteristic of members of the zinc-binding metalloprotease superfamily. Sequence comparisons with known enzymes of this class revealed that CD13 is identical to aminopeptidase N, a membrane-bound glycoprotein thought to be involved in the metabolism of regulatory peptides by diverse cell types, including small intestinal and renal tubular epithelial cells, macrophages, granulocytes, and synaptic membranes prepared from cells of the central nervous system.

Introduction

Normal human myeloid cells and their leukemic counterparts bear unique cell surface differentiation antigens which, by convention, are assigned to cluster differentiation (CD)[1] groups based on their specific reactivity with defined monoclonal antibodies (1). Antibodies used to identify the CD13 cluster group bind a 150-kD cell surface glycoprotein (gp150) that is expressed by committed granulocyte-monocyte progenitors (CFU-GM) and by cells of the granulocytic and monocytic lineages at all morphologically distinct stages of differentiation (2–5). CD13 is also expressed by leukemic blasts from a high percentage of patients with acute myeloid leukemia (2, 6–10) and from a smaller group of patients with acute lymphoid leukemia (10–12) who have an increased risk of treatment failure, especially when the lymphoblasts coexpress CD13 epitopes and B-lineage markers (11). This molecule appears to be highly immunogenic: 11 monoclonal antibodies independently derived from mice immunized with human myeloid cells bound CD13 epitopes in studies reported at a recent international workshop (1, 13). These antibodies are termed MY7, MCS-2, SJ-1D1, WM-15, CLB-mon-gran/2, MoU28, MoU48, DU-HL60-4, 22A5, 72a, and 124a4. CD13-specific antibodies do not bind to normal B or T lymphocytes but do react with nonhematopoietic cells, including fibroblasts, osteoclasts, and cells that line renal proximal tubules and bile duct canaliculi (1).

Biochemical studies have demonstrated that CD13-specific antibodies immunoprecipitate a 130-kD glycoprotein (gp130), as well as gp150, from human myeloid leukemic cell lines (14, 15). Metabolic labeling studies established that gp130 is an intracellular precursor of gp150, differing from the larger cell surface form of the molecule in the composition of its carbohydrate chains. When cells were labeled in the presence of tunicamycin, an antibiotic that blocks the addition of asparagine-linked oligosaccharide chains, a single unglycosylated polypeptide of 110 kD was immunoprecipitated (14, 15). A comparison of labeled tryptic cleavage products of gp130 and gp150 molecules confirmed that these glycoproteins have an identical primary structure and thus are posttranslationally modified products of a single gene (14).

Sequential rounds of DNA-mediated gene transfer and fluorescence-activated cell sorting were used to isolate the gene encoding CD13 in a mouse genetic background (14). Sequences from the CD13 locus that annealed to a human repeated sequence probe were cloned from a tertiary mouse cell transformant that had amplified the transfected human gene (16). Molecular subclones were then used to isolate the complete CD13 gene from a human placental genomic DNA library; the intact gene, assembled from three recombinant phages, encoded authentic gp150 glycoproteins when it was transfected into mouse fibroblasts. Probes prepared from subclones within the CD13 locus were used to identify a 4.0-kb RNA transcript expressed by human myeloid cells and to assign the CD13 gene to the distal long arm of chromosome 15.

We now report the isolation and characterization of overlapping cDNA clones which together contain all of the CD13 coding sequences. The deduced primary structure and NH_2-

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1. *Abbreviations used in this paper:* CALLA, common acute lymphoblastic leukemia antigen; CD, cluster of differentiation; gp150, 150-kD cell surface glycoprotein (CD13); gp130, the 130-kD precursor of gp150.

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terminal amino acid sequence of gp150 indicate that it is an integral membrane protein with a large extracellular carboxy-terminal domain, a short intracellular amino-terminal segment, and a hydrophobic signal sequence that is retained and functions as the transmembrane domain. Moreover, the predicted amino acid sequence of CD13 is virtually identical to the recently determined sequence of aminopeptidase N, a prominent intrinsic enzyme of the brush border membranes of the small intestine and renal proximal tubules.

Methods

cDNA cloning and DNA sequence analysis. We previously identified restriction fragments lacking human repetitive sequences that are included in the CD13 genomic locus and that anneal to a 4.0-kb RNA transcript expressed by the HL-60 and KG1 human myeloid leukemic cell lines (16). A mixture of these fragments was nick-translated and used as a probe to screen an oligo-dT primed lambda gt11 cDNA library prepared with polyadenylated RNA from HL-60 cells (a gift of Dr. Michael Holers, Washington University, St. Louis, MO; 17). Additional clones were isolated from a cDNA library prepared in the lambda ZAP vector (Stratagene Corp., La Jolla, CA) with polyadenylated RNA from KG1 cells. First strand synthesis was initiated with random primers and with synthetic oligonucleotides inversely complementary to 5′ sequences of clones isolated from the oligo-dT primed library.

Eco RI inserts from the lambda gt11 clones were subcloned into the pBluescript plasmid (Stratagene); lambda ZAP phages were rescued directly as recombinant pBluescript plasmids with procedures recommended by the manufacturer. Restriction endonuclease sites were mapped in the subcloned cDNA inserts, and restriction fragments were nick-translated and used as probes on Southern blots of digested DNA from both cDNA and genomic phage clones (16). In this way, the cDNA clones were oriented relative to each other and to the CD13 genomic locus. To determine the 5′ to 3′ orientation of the cDNA clones relative to messenger RNA, single-stranded RNA probes were prepared from selected cDNA inserts subcloned in pBluescript. Probes were transcribed from each strand of the cDNA insert with the T3- and T7-specific polymerases that bind specific promoter sequences flanking the cloning sites of this vector, using a kit supplied by Promega Biotec (Madison, WI). Each probe was hybridized to Northern blots, prepared as described previously (16), to determine which probe orientation was complementary to the 4.0-kb CD13 messenger RNA.

Based on an extensive map of restriction endonuclease sites within the inserts of the three longest cDNA clones (λ6, λ29 and λ71), a series of restriction fragments were subcloned into the M13mp18 and M13mp19 vectors for DNA sequence analysis by the Sanger dideoxy chain-termination method (18) using the Sequenase DNA sequencing kit (United States Biochemical Corp, Cleveland, OH). The entire cDNA was sequenced on both strands and each cloning site was resequenced in overlapping subclones. The sequence was verified for each subclone by using fluorescent dye-labeled primers and the 370A automated DNA sequencer (Applied Biosystems). IntelliGenetics (Mountain View, CA) software was used for all sequence manipulation and analysis.

Expression of cDNA sequences cloned in a retroviral vector. Full length CD13 cDNA was reconstructed by subcloning the 5′ 1.8 kb Eco RI insert of λ29 (see Fig. 1) into a pBluescript plasmid containing the 3′ 1.6 kb Eco RI insert of λ6, which had been linearized by partial digestion with Eco RI. The restriction sites in the resulting plasmids were mapped to identify a plasmid that contained both Eco RI cDNA restriction fragments ligated in the correct 5′-3′ orientation. Sequencing analysis subsequently disclosed a 139-bp deletion in the insert of λ29, by comparison with the corresponding sequence of λ71 and other clones spanning this region. This deletion interrupted the CD13 open reading frame, and presumably represented a cloning artifact. Therefore, a 1.0-kb Nco I restriction fragment containing this region from λ71 was substituted for the corresponding restriction fragment in the plasmid containing the reconstructed cDNA. The resulting pBluescript plasmid was digested with Sal I, which cut only in the cloning polylinker adjacent to the 5′ end of the cDNA and with Xba I, which cut once in the cDNA insert in the 3′ untranslated region upstream of the polyadenylation signal (see Fig. 2). The resulting 3.4-kb restriction fragment, which contained all of the predicted CD13 coding sequences, was inserted into the unique Bam HI site of the murine retroviral vector pZIP*neo*SV(X)-1 (19) provided by Dr. Richard C. Mulligan, Whitehead Institute, Boston, MA. Blunt end ligation was employed, after the recessed 3′ ends of both the insert and the vector had been filled in with the Klenow fragment of *Escherichia coli* polymerase I. Colonies containing recombinant plasmids were identified by hybridization, and a plasmid with the correct 5′ to 3′ orientation of the cDNA insert relative to the vector was selected on the basis of restriction endonuclease mapping.

This construction linked the CD13 gene to DNA sequences of the vector encoding neomycin resistance (*neo*); the gp150 protein is translated from the unspliced retroviral RNA, whereas the *neo* product is synthesized from a spliced mRNA. The retroviral construct was transfected by the calcium phosphate method into NIH-3T3 cells (14). After transfection, the cells were cultured in medium containing 800 μg/ml of G418 (Geneticin; Gibco Laboratories, Grand Island, NY), to select for expression of the *neo* gene. G418-resistant cells were sorted by flow cytometry after they had been stained with the monoclonal antibody MY7 (Coulter Immunology, Hialeah, FL), which reacts with an epitope of gp150 (2, 14). Transfected cells isolated according to their reactivity with MY7 were tested by flow cytometry for binding of other monoclonal antibodies that react with epitopes of CD13: SJ-1D1 (20) from Dr. Joseph Mirro of St. Jude Children's Research Hospital, Memphis, TN; and MCS-2 (15) from Dr. Jun Minowada of Loyola University, Chicago, IL. Indirect immunofluorescence labeling, flow cytometric analysis, and cell sorting were performed as previously described (14).

Metabolic labeling and immunoprecipitation. Subconfluent cultures of cells were incubated at 37°C in 5 ml methionine-free DMEM (Gibco Laboratories) containing 5% dialyzed fetal calf serum for 30 min and then labeled for 60 min in 2 ml of the same medium containing 0.25 mCi of [^{35}S]methionine (1,200 Ci/mmol) per ml. An equal volume of complete medium was added, and the incubation was continued for an additional 60 min. The cells were rinsed in ice-cold PBS and lysed in RIPA buffer (50 mM Tris hydrochloride [pH 7.4], 150 mM NaCl, 20 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) containing 2% aprotinin and 1% PMSF to inhibit proteases. The lysates were immunoprecipitated and separated by SDS-PAGE (14, 21); the mobilities of radiolabeled proteins were determined by autoradiography relative to known polypeptide standards.

Immunoprecipitation of glycoproteins for NH_2-terminal protein sequence analysis. Exponentially growing HL-60 cells (1×10^9) were lysed in RIPA buffer and immunoprecipitated with antiserum specific for CD13 epitopes. For this immunoprecipitation, we used a CD13-specific polyvalent antiserum produced by neonatal (8-d-old) NFS mice that are syngeneic with NIH-3T3 cells. Tumorigenic cells from the NIH-3T3 cell tertiary transformant SJ-150-B (16, 22), which coexpresses high levels of CD13 together with the transforming glycoprotein encoded by the v-*fms* oncogene, were injected subcutaneously into the mice. The animals with tumors were bled, and pooled sera were tested for their ability to react with CD13 in immunofluorescence and immunoprecipitation assays. As we had previously observed for similar antisera produced against epitopes of CD33 (23), pooled CD13-specific antiserum proved superior to monoclonal antibodies for precipitating large quantities of the gp130 and gp150 glycoproteins, presumably because it recognizes multiple epitopes with high affinity. The immunoprecipitates were separated by SDS-PAGE, and gp150 and its gp130 precursor were transferred to Immobilon membranes (Miles Laboratories, Elkhart, IN) following methods of Matsudaira (24), and visualized by Coomassie blue staining. The NH_2-terminal amino acid sequences of both the gp130 and gp150 forms of the molecule were determined from two independent preparations according to established procedures (24, 25).

Results

Isolation of CD13 cDNA clones. Unique sequence probes derived from the CD13 genomic locus were used to screen cDNA libraries that were prepared with polyadenylated RNA extracted from human myeloid cell lines that express CD13. 12 strongly hybridizing clones were isolated from a cDNA library primed with oligo-dT, and 10 additional clones were obtained from a library primed with a mixture of random primers and specific oligonucleotides representing sequences inversely complementary to 5′ CD13 sequences. Restriction endonuclease cleavage sites were determined for the three clones with the largest inserts; defined restriction fragments were then used as probes in Southern blotting experiments to align the cDNA clones and to establish their orientation relative to previously isolated genomic clones (Fig. 1). Together, the overlapping cDNA clones spanned 3.5 kb of the CD13 messenger RNA, which was estimated to be 4.0 kb in length as judged from Northern blots hybridized with the same genomic probes that were used to isolate the cDNA clones (16).

When used as probes for Southern blots of restriction digests of previously isolated genomic clones, the cDNA restriction fragments hybridized to a region of ~ 20 kb (Fig. 1). Genomic clones containing this region mediate expression of gp150 when appropriately ligated together and transfected into mouse fibroblasts (16). The 5′ to 3′ orientation of the cDNA clones shown in Fig. 1 was determined by hybridizing strand-specific RNA probes prepared from cDNA restriction fragments subcloned in the pBluescript transcription vector to Northern blots of polyadenylated RNA from myeloid cell lines. RNA probes prepared from restriction fragments cloned in an antisense orientation hybridized to the 4.0 kb CD13 messenger RNA (data not shown).

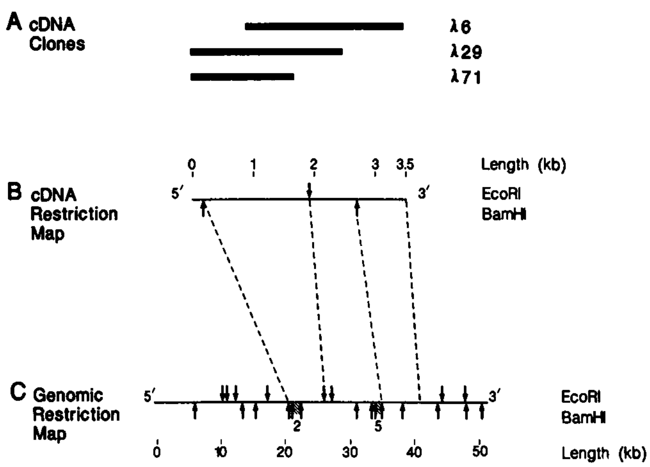

Figure 1. Restriction map of CD13 cDNA clones (*A* and *B*), in relation to the restriction map of the previously isolated genomic locus (*C*). Clones λ6 and λ29 were isolated from an oligo-dT primed cDNA library made from HL-60 cell mRNA, using a probe prepared from a mixture of two unique sequence genomic restriction fragments (labeled 2 and 5, *C*) that had been previously shown to hybridize with the 4.0 kb CD13 mRNA. Clone λ71 was isolated from a cDNA library prepared from KG1 cell mRNA by using a mixture of random primers and oligonucleotides inversely complementary to 5′ sequences from λ29. The inserts of these clones contained one internal Eco RI site and two Bam HI sites, with positions as shown in *B*. The corresponding restriction sites in the CD13 genomic map (*dashed lines* connecting *B* with *C*) were identified with cDNA probes from Bam HI-Eco RI restriction fragments of the inserts of λ6 and λ29 to probe Southern blots of restriction digests of previously isolated phage clones that span the CD13 genomic locus (16).

CD13 cDNA sequence. The complete nucleotide sequence of overlapping CD13 cDNA inserts contained in the λ6, λ29, and λ71 clones extends for 3,494 nucleotides and is flanked by a poly(A) sequence at its 3′ end (Fig. 2). The longest predicted open reading frame of 2901 residues (nucleotides 121-3021) is preceded by 120 nucleotides of 5′ untranslated sequence and terminates with 473 nucleotides of 3′ untranslated sequence. A polyadenylation signal (AATAAA) (26) is located 13 nucleotides upstream from the poly(A) sequence at the 3′ end of the cDNA. The first ATG codon, at position 121, matches the consensus sequence for a translation initiation site (27). Seven residues separate the initiator methionine from a hydrophobic region of 24 amino acids, which is likely to represent a signal peptide sequence necessary for transport of the nascent polypeptide chains into the lumen of the endoplasmic reticulum. The deduced amino acid sequence of the CD13 polypeptide predicts a total of 967 amino acids with a molecular mass of 110 kD, in agreement with the apparent mass of the unglycosylated CD13 polypeptide synthesized by cells grown in the presence of tunicamycin (14, 15). 11 canonical sites (Asn-X-Ser/Thr) for the addition of asparagine-linked oligosaccharide chains are present 3′ to the putative membrane-spanning segment. Nearly all of these potential sites may be utilized, because each high-mannose chain has an estimated mass of 2.5 kD, and immature gp130 precursor molecules differ by ~ 20 kD from the mass of the unglycosylated polypeptide. Five cysteine residues are distributed over the polypeptide sequence. Because the CD13 mRNA is estimated to be 4.0 kb by Northern blot analysis, and our cDNA clones span 3494 nucleotides, the native transcripts most likely contain additional untranslated nucleotides at their 5′ ends.

The entire CD13 cDNA and protein sequences were used to search for similarity with other sequences available from the NIH DNA sequence library (GenBank; Release 56), the European Molecular Biology Laboratory DNA sequence library (EMBL; Release 14), and the National Biomedical Research Foundation protein sequence library (NBRF; Release 16). These searches did not disclose statistically significant similarity between the sequence obtained for CD13 and other previously sequenced proteins or DNA sequences. We did, however, identify a region spanning amino acid residues 388-392 that conforms to a pentapeptide consensus sequence, His-Glu-[Ile, Leu, Met]-X-His, which is essential for zinc coordination and the catalytic activity of metalloproteases (27–31).

Sequence comparisons with known enzymes of this class revealed virtual identity between our CD13 sequence and the recently determined sequence of aminopeptidase N (32). Our CD13 cDNA clone includes 96 nucleotides at its 5′ end and 10 nucleotides plus the poly(A) sequence at its 3′ end that are missing from the aminopeptidase N clone. Within the sequenced regions of both cDNA clones, there were 10 nucleotide mismatches, only four of which result in predicted amino

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1     TAATTTTTGC CCAGTCTGCC TGTTGTGGGG CTCCTCCCCT TTGGGGATAT AAGCCCGGCC TGGGGCTGCT CCGTTCTCTG CCTGGCCTGA GGCTCCCTGA GCCGCCTCCC CACCATCACC

121   ATG GCC AAG GGC TTC TAT ATT TCC AAG TCC CTG GGC ATC CTG GGG ATC CTC CTG GGC GTG GCA GCC GTG TGC ACA ATC ATC GCA CTG TCA GTG GTG TAC TCC CAG GAG AAG AAC AAG AAC
1     MET Ala Lys Gly Phe Tyr Ile Ser Lys Ser Leu Gly Ile Leu Gly Ile Leu Leu Gly Val Ala Ala Val Cys Thr Ile Ile Ala Leu Ser Val Val Tyr Ser Gln Glu Lys Asn Lys Asn

241   GCC AAC AGC TCC CCC GTG GCC TCC ACC ACC CCG TCC GCC TCA GCC ACC ACC AAC CCC GCC TCG GCC ACC ACC TTG GAC CAA AGT AAA GCG TGG AAT CGT TAC CGC CTC CCC AAC ACG CTG
41    Ala Asn Ser Ser Pro Val Ala Ser Thr Thr Pro Ser Ala Ser Ala Thr Thr Asn Pro Ala Ser Ala Thr Thr Leu Asp Gln Ser Lys Ala Trp Asn Arg Tyr Arg Leu Pro Asn Thr Leu
                          *
61    AAA CCC GAT TCC TAC CAG GTG ACG CTG AGA CCG TAC CTC ACC CCC AAT GAC AGG GGC CTG TAC GTT TTT AAG GGC TCC AGC ACC GTC CGT TTC ACC TGC AAG GAG GCC ACT GAC GTC ATC
81    Lys Pro Asp Ser Tyr Gln Val Thr Leu Arg Pro Tyr Leu Thr Pro Asn Asp Arg Gly Leu Tyr Val Phe Lys Gly Ser Ser Thr Val Arg Phe Thr [Cys] Lys Glu Ala Thr Asp Val Ile

481   ATC ATC CAC AGC AAG AAG CTC AAC TAC ACC CTC AGC CAG GGG CAC AGG GTG GTC CTG CGT GGT GTG GGA GGC TCC CAG CCC CCC GAC ATT GAC AAG ACT GAG CTG GTG GAG CCC ACC GAG
121   Ile Ile His Ser Lys Lys Leu Asn Tyr Thr Leu Ser Gln Gly His Arg Val Val Leu Arg Gly Val Gly Gly Ser Gln Pro Pro Asp Ile Asp Lys Thr Glu Leu Val Glu Pro Thr Glu

601   TAC CTG GTG GTG CAC CTC AAG GGC TCC CTG GTG AAG GAC AGC CAG TAT GAG ATG GAC AGC GAG TTC GAG GGG GAG TTG GCA GAT GAC CTG GCG GGC TTC TAC CGC AGC GAG TAC ATG GAG
161   Tyr Leu Val Val His Leu Lys Gly Ser Leu Val Lys Asp Ser Gln Tyr Glu Met Asp Ser Glu Phe Glu Gly Glu Leu Ala Asp Asp Leu Ala Gly Phe Tyr Arg Ser Glu Tyr Met Glu

721   GGC AAT GTC AGA AAG GTG GTG GCC ACT ACA CAG ATG CAG GCT GCA GAT GCC CGG AAG TCC TTC CCA TGC TTC GAT GAG CCG GCC ATG AAG GCC GAG TTC AAC ATC ACG CTT ATC CAC CCC
201   Gly Asn Val Arg Lys Val Val Ala Thr Thr Gln Met Gln Ala Ala Asp Ala Arg Lys Ser Phe Pro [Cys] Phe Asp Glu Pro Ala Met Lys Ala Glu Phe Asn Ile Thr Leu Ile His Pro

841   AAG GAC CTG ACA GCC CTG TCC AAC ATG CTT CCC AAA GGT CCC AGC ACC CCA CTT CCA GAA GAC CCC AAC TGG AAT GTC ACT GAG TTC CAC ACC ACG CCC AAG ATG TCC ACG TAC TTG CTG
241   Lys Asp Leu Thr Ala Leu Ser Asn Met Leu Pro Lys Gly Pro Ser Thr Pro Leu Pro Glu Asp Pro Asn Trp Asn Val Thr Glu Phe His Thr Thr Pro Lys Met Ser Thr Tyr Leu Leu

961   GCC TTC ATT GTC AGT GAG TTC GAC TAC GTG GAG AAG CAG GCA TCC AAT GGT GTC TTG ATC CGG ATC TGG GCC CGG CCC AGT GCC ATT GCG GCG GGC CAC GGC GAT TAT GCC CTG AAC GTG
281   Ala Phe Ile Val Ser Glu Phe Asp Tyr Val Glu Lys Gln Ala Ser Asn Gly Val Leu Ile Arg Ile Trp Ala Arg Pro Ser Ala Ile Ala Ala Gly His Gly Asp Tyr Ala Leu Asn Val

1081  ACG GGC CCC ATC CTT AAC TTC TTT GCT GGT CAT TAT GAC ACA CCC TAC CCA CTC CCA AAA TCA GAC CAG ATT GGC CTG CCA GAC TTC AAC GCC GGC GCC ATG GAG AAC TGG GGA CTG GTG
321   Thr Gly Pro Ile Leu Asn Phe Phe Ala Gly His Tyr Asp Thr Pro Tyr Pro Leu Pro Lys Ser Asp Gln Ile Gly Leu Pro Asp Phe Asn Ala Gly Ala Met Glu Asn Trp Gly Leu Val

1201  ACC TAC CGG GAG AAC TCC CTG CTG TTC GAC CCC CTG TCC TCC TCC AGC AGC AAC AAG GAG CGG GTG GTC ACT GTG ATT GCT CAT GAG CTG GCC CAC CAG TGG TTC GGG AAC CTG GTG ACC
361   Thr Tyr Arg Glu Asn Ser Leu Leu Phe Asp Pro Leu Ser Ser Ser Ser Ser Asn Lys Glu Arg Val Val Thr Val Ile Ala His Glu Leu Ala His Gln Trp Phe Gly Asn Leu Val Thr
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1321  ATA GAG TGG TGG AAT GAC CTG TGG CTG AAC GAG GGC TTC GCC TCC TAC GTG GAG TAC CTG GGT GCT GAC TAT GCG GAG CCC ACC TGG AAC TTG AAA GAC CTC ATG GTG CTG AAT GAT GTG
401   Ile Glu Trp Trp Asn Asp Leu Trp Leu Asn Glu Gly Phe Ala Ser Tyr Val Glu Tyr Leu Gly Ala Asp Tyr Ala Glu Pro Thr Trp Asn Leu Lys Asp Leu Met Val Leu Asn Asp Val

1441  TAC CGC GTG ATG GCA GTG GAT GCA CTG GCC TCC TCC CAC CCG CTG TCC ACA CCC GCC TCG GAG ATC AAC ACG CCG GCC CAG ATC AGT GAG CTG TTT GAC GCC ATC TCC TAC AGC AAG GGC
441   Tyr Arg Val Met Ala Val Asp Ala Leu Ala Ser Ser His Pro Leu Ser Thr Pro Ala Ser Glu Ile Asn Thr Pro Ala Gln Ile Ser Glu Leu Phe Asp Ala Ile Ser Tyr Ser Lys Gly

1561  GCC TCA GTC CTC AGG ATG CTC TCC AGC TTC CTG TCC GAG GAC GTA TTC AAG CAG GGC CTG GCG TCC TAC CTC CAC ACC TTT GCC TAC CAG AAC ACC ATC TAC CTG AAC CTG TGG GAC CAC
481   Ala Ser Val Leu Arg Met Leu Ser Ser Phe Leu Ser Glu Asp Val Phe Lys Gln Gly Leu Ala Ser Tyr Leu His Thr Phe Ala Tyr Gln Asn Thr Ile Tyr Leu Asn Leu Trp Asp His
                                                            *
1681  CTG CAG GAG GCT GTG AAC AAC CGG TCC ATC CAA CTC CCC ACC ACC GTG CGG GAC ATC ATG AAC CGC TGG ACC CTG CAG ATG GGC TTC CCG GTC ATC ACG GTG GAT ACC AGC ACG GGG ACC
521   Leu Gln Glu Ala Val Asn Asn Arg Ser Ile Gln Leu Pro Thr Thr Val Arg Asp Ile Met Asn Arg Trp Thr Leu Gln Met Gly Phe Pro Val Ile Thr Val Asp Thr Ser Thr Gly Thr

1801  CTT TCC CAG GAG CAC TTC CTC CTT GAC CCC GAT TCC AAT GTT ACC CGC CCC TCA GAA TTC AAC TAC GTG TGG ATT GTG CCC ATC ACA TCC ATC AGA GAT GGC AGA CAG CAG CAG GAC TAC
561   Leu Ser Gln Glu His Phe Leu Leu Asp Pro Asp Ser Asn Val Thr Arg Pro Ser Glu Phe Asn Tyr Val Trp Ile Val Pro Ile Thr Ser Ile Arg Asp Gly Arg Gln Gln Gln Asp Tyr
              *
1921  TGG CTG ATA GAT GTA AGA GCC CAG AAC GAT CTC TTC AGC ACA TCA GGC AAT GAG TGG GTC CTG CTG AAC CTC AAT GTG ACG GGC TAT TAC CGG GTG AAC TAC GAC GAA GAG AAC TGG AGG
601   Trp Leu Ile Asp Val Arg Ala Gln Asn Asp Leu Phe Ser Thr Ser Gly Asn Glu Trp Val Leu Leu Asn Leu Asn Val Thr Gly Tyr Tyr Arg Val Asn Tyr Asp Glu Glu Asn Trp Arg

2041  AAG ATT CAG ACT CAG CTG CAG AGA GAC CAC TCG GCC ATC CCT GTC ATC AAT CGG GCA CAG ATC ATT AAT GAC GCC TTC AAC CTG GCC AGT GCC CAT AAG GTC CCT GTC ACT CTG GCG CTG
641   Lys Ile Gln Thr Gln Leu Gln Arg Asp His Ser Ala Ile Pro Val Ile Asn Arg Ala Gln Ile Ile Asn Asp Ala Phe Asn Leu Ala Ser Ala His Lys Val Pro Val Thr Leu Ala Leu

2161  AAC AAC ACC CTC TTC CTG ATT GAA GAG AGA CAG TAC ATG CCC TGG GAG GCC GCC CTG AGC AGC CTG AGC TAC TTC AAG CTC ATG TTT GAC CGC TCC GAG GTC TAT GGC CCC ATG AAG AAC
681   Asn Asn Thr Leu Phe Leu Ile Glu Glu Arg Gln Tyr Met Pro Trp Glu Ala Ala Leu Ser Ser Leu Ser Tyr Phe Lys Leu Met Phe Asp Arg Ser Glu Val Tyr Gly Pro Met Lys Asn

2281  TAC CTG AAG AAG CAG GTC ACA CCC CTC TTC ATT CAC TTC AGA AAT AAT ACC AAC AAC TGG AGG GAG ATC CCA GAA AAC CTG ATG GAC CAG TAC AGC GAG GTT AAT GCC ATC AGC ACC GCC
721   Tyr Leu Lys Lys Gln Val Thr Pro Leu Phe Ile His Phe Arg Asn Asn Thr Asn Asn Trp Arg Glu Ile Pro Glu Asn Leu Met Asp Gln Tyr Ser Glu Val Asn Ala Ile Ser Thr Ala

2401  TGC TCC AAC GGA GTT CCA GAG TGT GAG GAG ATG GTC TCT GGC CTT TTC AAG CAG TGG ATG GAG AAC CCC AAT AAT AAC CCG ATC CAC CCC AAC CTG CGG TCC ACC GTC TAC TGC AAC GCT
761   [Cys] Ser Asn Gly Val Pro Glu [Cys] Glu Glu Met Val Ser Gly Leu Phe Lys Gln Trp Met Glu Asn Pro Asn Asn Asn Pro Ile His Pro Asn Leu Arg Ser Thr Val Tyr [Cys] Asn Ala

2521  ATC GCC CAG GGC GGG GAG GAG GAG TGG GAC TTC GCC TGG GAG CAG TTC CGA AAT GCC ACA CTG GTC AAT GAG GCT GAC AAG CTC CGG GCA GCC CTG GCC TGC AGC AAA GAG TTG TGG ATC
801   Ile Ala Gln Gly Gly Glu Glu Glu Trp Asp Phe Ala Trp Glu Gln Phe Arg Asn Ala Thr Leu Val Asn Glu Ala Asp Lys Leu Arg Ala Ala Leu Ala [Cys] Ser Lys Glu Leu Trp Ile

2641  CTG AAC AGG TAC CTG AGC TAC ACC CTG AAC CCG GAC TTA ATC CGG AAG CAG GAC GCC ACC TCT ACC ATC ATC AGC ATT ACC AAC AAC GTC ATT GGG CAA GGT CTG GTC TGG GAC TTT GTC
841   Leu Asn Arg Tyr Leu Ser Tyr Thr Leu Asn Pro Asp Leu Ile Arg Lys Gln Asp Ala Thr Ser Thr Ile Ile Ser Ile Thr Asn Asn Val Ile Gly Gln Gly Leu Val Trp Asp Phe Val
                              *
2761  CAG AGC AAC TGG AAG AAG CTT TTT AAC GAT TAT GGT GGT GGC TCG TTC TCC TTC TCC AAC CTC ATC CAG GCA GTG ACA CGA CGA TTC TCC ACC GAG TAT GAG CTG CAG CAG CTG GAG CAG
881   Gln Ser Asn Trp Lys Lys Leu Phe Asn Asp Tyr Gly Gly Gly Ser Phe Ser Phe Ser Asn Leu Ile Gln Ala Val Thr Arg Arg Phe Ser Thr Glu Tyr Glu Leu Gln Gln Leu Glu Gln

2881  TTC AAG AAG GAC AAC GAG GAA ACA GGC TTC GGC TCA GGC ACC CGG GCC CTG GAG CAA GCC CTG GAG AAG ACG AAA GCC AAC ATC AAG TGG GTG AAG GAG AAC AAG GAG GTG GTG CTC CAG
921   Phe Lys Lys Asp Asn Glu Glu Thr Gly Phe Gly Ser Gly Thr Arg Ala Leu Glu Gln Ala Leu Glu Lys Thr Lys Ala Asn Ile Lys Trp Val Lys Glu Asn Lys Glu Val Val Leu Gln
                                                   **         **
3001  TGG TTC ACA GAA AAC AGC AAA TAGTCCCCA GCCCTTGAAG TCACCCGGCC CCGATGCAAG GTGCCCACAT GTGTCCATCC CAGCGGCTGG TGCAGGGCCT CCATTCCTGG AGCCCGAGGC
961   Trp Phe Thr Glu Asn Ser Lys

3121  ACCAGTGTCC TCCCCTCAAG GACAAAGTCT CCAGCCCACG TTCTCTCTGC CTGTGAGCCA GTCTAGTTCC TGATGACCCA GGCTGCCTGA GCACCTCCCA GCCCCTGCCC CTCATGCCAA
                                                                      *
3241  CCCCGCCCTA GGCCTGGCAT GGCACCTGTC GCCCAGTGCC CTGGGGCTGA TCTCAGGGAA GCCCAGCTCC AGGGCCAGAT GAGCAGAAGC TCTCGATGGA CAATGAACGG CCTTGCTGGG
                                                                                                                  *
3361  GGCCGCCCTG TACCCTCTTT CACCTTTCCC TAAAGACCCT AAATCTGAGG AATCAACAGG GCAGCAGATC TGTATATTTT TTTCTAAGAG AAAATGTAAA TAAAGGATTT CTAGATGAAA

3481  AAAAAAAAAA AAAA
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Figure 2. Nucleic acid sequence and deduced amino acid sequence of human CD13 cDNA. Amino acids are numbered starting at the initial methionine of the CD13 polypeptide. The hydrophobic region that functions as both a signal peptide and single transmembrane region is denoted by a double underline. The penta-peptide consensus sequence for the zinc coordinating and catalytic domain of metalloproteases (His-Glu-Leu-Ala-His) is underlined (*dots*). Potential sites of NH_2-linked glycosylation are also underlined (*solid*), and cysteine residues are enclosed in boxes. The AATAAA box close to the polyadenylated 3′ end of the cDNA is overlined. There are 10 nucleotide mismatches between the CD13 and aminopeptidase N (32) sequences, indicated by asterisks.

acid differences (Fig. 2). Accordingly, Gln[86] in the CD13 sequence is predicted to be Arg[86] by the aminopeptidase N sequence, Val[536] to be Glu[536], Ile[603] to be Met[603], and Leu[887] to be Pro[887]. These differences could result from naturally occurring polymorphisms or errors introduced by reverse transcriptase during cDNA synthesis.

Expression of CD13 cDNA cloned in a retroviral vector. To confirm that the cDNA clones encoded bona fide gp150 mole-

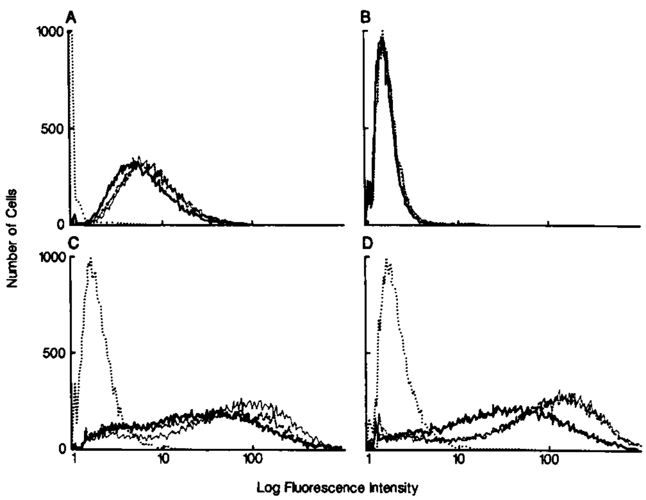

Figure 3. Expression of CD13 on the surface of NIH-3T3 cells transfected with CD13 cDNA sequences cloned in a retroviral vector. The flow cytometric profiles were obtained after binding of monoclonal antibodies specific for CD13 epitopes to HL-60 human myeloid cells (*A*), untransfected NIH-3T3 cells (*B*), and cells from two representative NIH-3T3 cultures that had been transfected with complete CD13 cDNA coding sequences (*C* and *D*) reconstructed from portions of the inserts of three overlapping cDNA clones. Transfected cells were grown in G418 to select for cells expressing the *neo* gene of the retroviral vector and then sorted once for the 2% of cells with the brightest fluorescence after staining with the CD13-specific monoclonal antibody, MY7. Each panel shows fluorescence profiles that resulted from testing the cells with the CD13-specific monoclonal antibodies MY7 (*dark solid line*), SJ-1D1 (*dashed line*), and MCS.2 (*light solid line*), compared with results with a mouse myeloma protein control (*dotted line*).

cules, we assembled the intact coding sequences from overlapping clones and inserted them into the murine retroviral vector pZIP*neo*SV(X)-1 (19). The retroviral construct was transfected into NIH-3T3 cells, and the cells were cultured in medium containing G418 to select for transfectants that expressed the *neo* gene contained in the vector. Analysis of these cells by flow cytometry after immunofluorescence labeling with the CD13-specific monoclonal antibody MY7 disclosed that over half the cells expressed high levels of gp150 at the cell surface. Cells that bound the highest levels of antibody were isolated by cell sorting, and cultured for further analysis. Fig. 3 shows the results of flow cytometric analysis of cells from two independently transfected cultures stained with three different CD13-specific monoclonal antibodies. The expression of gp150 epitopes by cells transfected with the retroviral vector was approximately tenfold higher than that observed for the HL-60 human myeloid leukemia cell line.

To characterize the biochemical properties of CD13 molecules expressed by cells transfected with the retroviral construct, we metabolically labeled cells with [^{35}S]methionine, and immunoprecipitated detergent lysates with the MY7 monoclonal antibody. High levels of polypeptides with the mobilities of gp130 and gp150 were detected in lysates from control HL-60 cells, in a tertiary mouse cell transformant (SJ-150-B) known to express high levels of gp150 (16, 22), and in three NIH-3T3 cultures that had been transfected with the CD13 retroviral vector (Fig. 4). No specifically precipitable polypeptide was observed in NIH-3T3 cells that were either untransfected or transfected with a plasmid containing the v-*fms* oncogene. Immature gp130 molecules had identical apparent mobilities in human HL-60 cells and in transfected mouse cells; the more rapid mobility of the mature cell surface form of the molecule in mouse cells, as compared with HL-60 cells, has been observed previously (14, 16) and may reflect differences in glycosylation in the different cell types.

Topology of CD13. The hydrophobicity profile predicted by the algorithm of Kyte and Doolittle (33) showed a 24 amino acid hydrophobic region, starting nine amino acids from the amino terminus, that could function as both a signal peptide and a transmembrane domain (Fig. 1). No other hydrophobic region that would be of sufficient length to span the plasma membrane was identified. This suggested that CD13 might be a member of a small family of integral membrane proteins, in which the signal sequence is not removed after translocation of the NH_2-terminus into the lumen of the endoplasmic reticulum, but rather is retained to serve as a transmembrane anchor (34). Proteins of this type are oriented with their amino terminus in the cytoplasm and their carboxyl terminus outside the cell.

To test this hypothesis, we determined the NH_2-terminal peptide sequence of both the gp130 and gp150 forms of the molecule, which were immunoprecipitated with specific antisera from lysates of HL-60 cells. The yields indicated that ~ 10% of the molecules were susceptible to Edman degradation, and the first 20 residues matched amino acids 2-21 of the

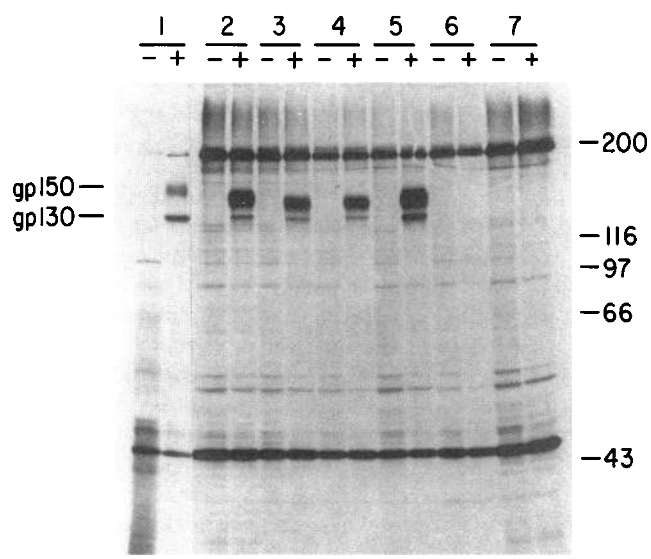

Figure 4. Biochemical characterization of gp130 and gp150 glycoproteins expressed by NIH-3T3 cells transfected with CD13 cDNA sequences cloned in a retroviral vector. Cells were metabolically labeled with [^{35}S]methionine for 1 h and incubated an additional hour with complete medium; then, detergent lysates were immunoprecipitated with the MY7 CD13-specific monoclonal antibody (+) or with a control monoclonal antibody (–). Labeled proteins in immune complexes were analyzed in polyacrylamide gels containing SDS. Results are shown for HL-60 cells (*lane 1*); the tertiary transformant SJ-150-B (16, 22), derived from serial transfections of genomic DNA with the v-*fms* oncogene as a selectable marker (*2*); NIH-3T3 cells from three cultures independently transfected with complete CD13 coding sequences cloned in a retroviral vector (*3, 4,* and *5*); untransfected NIH-3T3 cells (*6*); and NIH-3T3 cells transfected with the v-*fms* oncogene (*7*).

sequence predicted from the CD13 cDNA nucleotide sequence (Ala-Lys-Gly-Phe-Tyr-Ile-Ser-Lys-Ser-Leu-Gly-Ile-Leu-Gly-Ile-Leu-Leu-Gly-Val-Ala; Fig. 2). The N-terminal amino acid sequence is consistent with translation being initiated at the ATG codon predicted from the nucleic acid sequence, and indicates that the signal sequence is not cleaved after insertion of the molecules into membranes of the endoplasmic reticulum. This result is typical of integral membrane proteins whose signal sequences are retained as a transmembrane anchor. In such proteins, the amino-terminal methionine is characteristically removed, and the second residue is acetylated, leaving only a minority of unblocked amino termini that remain susceptible to Edman degradation (35–37).

Discussion

The cellular distribution and biochemical properties of CD13 glycoproteins suggested that they might have important physiologic roles, not only on myeloid cells but also on cells from the diverse tissues that bind CD13-specific monoclonal antibodies (1, 14, 15). We undertook the molecular cloning of CD13 cDNA with the idea that the deduced primary structure of the polypeptide would provide insight into the functions of this glycoprotein on normal and malignant cells. The translated amino acid sequence predicts a polypeptide of 967 amino acids with 11 potential sites of asparagine-linked oligosaccharide addition, accounting for the 110-kD molecular mass of the unglycosylated polypeptide and the additional mass attributable to the carbohydrate moiety of the cotranslationally modified glycoprotein (14, 15). Aminoterminal protein sequence analysis indicated that CD13 molecules are synthesized with an uncleaved signal sequence and that proteolytic processing at the amino-terminus is limited to removal of the initiator methionine. Because the retained signal sequence is the only potential membrane-spanning segment apparent from hydrophobicity analysis, CD13 glycoproteins are likely to be oriented with their amino terminus inside and their carboxyl terminus outside the cell. Integral membrane proteins with this topology include a small group of molecules that function predominately as receptors or membrane-bound enzymes (34–40). The extracellular carboxyterminal CD13 domain contains a pentapeptide signature sequence that serves as the catalytic site of zinc-binding metalloproteases, suggesting that CD13 is a membrane-bound enzyme of this class (27–31). Comparison of the recently published sequence of a cDNA clone encoding aminopeptidase N (32), a prominent metalloprotease, with our CD13 cDNA sequence disclosed that the two molecules are identical.

Aminopeptidase N (EC 3.4.11.2) is an important enzyme of the brush border membranes of the small intestine, renal proximal tubules, and placenta (41–43). Its expression has also been documented on synaptic membranes of the central nervous system (44) and on the surface of macrophages (45) and granulocytes (46, 47). This enzyme catalyzes the removal of NH_2-terminal amino acids from peptides, with a preference for neutral residues, but with broad specificity in the cleavage of basic and acidic residues as well (48). The natural substrates appear to be peptides rather than proteins, but the enzyme is more effective in the removal of residues from oligopeptides than from dipeptides (48). In the intestinal brush border where aminopeptidase N constitutes 8% of the total protein, the carboxyterminal enzymatic domain faces the lumen and most likely plays an important role in the final stages of the digestion of small peptides (41, 42). In other tissues, the enzyme has been postulated to function in the hydrolytic inactivation of regulatory peptides, including enkephalins, that are involved in signal transduction at the cell membrane (44, 45, 49).

The synthesis and posttranslational processing of aminopeptidase N have been characterized biochemically in epithelial cells from the small intestine and renal proximal tubules of the pig (50, 51). Pulse-chase experiments have demonstrated that newly synthesized molecules have an apparent molecular mass of ~ 140 kD and contain cotranslationally added asparagine-linked oligosaccharide chains that are rich in mannose. Within 30 to 60 min of synthesis, the side chains are remodeled to complex oligosaccharides, resulting in the 160 kD mature form of the molecule that is expressed at the cell surface. Endoglycosidase H treatment of the immature form of the molecule removes the high-mannose oligosaccharide chains, and produces a polypeptide backbone of 115 kD. These results obtained by two different laboratories studying epithelial cells from separate organs of the pig, are perfectly consistent with our studies of CD13 synthesis and processing by human myeloid leukemia cell lines (14). In addition, aminopeptidase N has been shown to be expressed as a homodimer on the surface of intestinal epithelial cells of several species, including rat, pig, and human, but as a monomer in the rabbit (42, 43).

The gene encoding the common acute lymphoblastic leukemia antigen (CALLA or CD10) was recently cloned (31, 35), and its cDNA sequence was shown by Letarte and co-workers (31) to be identical to that of neutral endopeptidase (EC 3.4.24.11), a membrane-associated enzyme also known as metalloendopeptidase or enkephalinase (28, 52, 53). The predicted amino acid sequences of CD13/aminopeptidase N and CD10/neutral endopeptidase do not show significant overall similarity; however, both molecules are integral membrane metalloproteases with the characteristic zinc-binding motif in their extracellular carboxyterminal domains (27–31). Neutral endopeptidase hydrolyzes peptide bonds at the amino side of hydrophobic amino acids (54), and is also thought to inactivate regulatory peptides at the cell surface (55–61). Both of these enzymes are expressed on epithelial cells of the renal proximal tubule and small intestine, granulocytes, stromal cells, and synaptic membranes in the central nervous system (1, 55–72). The two membrane-bound enzymes collaborate in the hydrolysis of oligopeptides in the small intestine (41, 42) and appear to act in concert to inactivate opioid peptides and enkephalins in the brain (44, 49), as well as tuftsin (46, 47) and the chemotactic peptide FMLP by neutrophils (60, 61). On hematopoietic cells, there are clear differences in their patterns of expression: CD10/neutral endopeptidase is uniquely expressed by early lymphoid precursors (62–65), while monocytes and committed myeloid progenitors express only CD13/aminopeptidase N (1–10). Neither enzyme is expressed by mature B or T lymphocytes. The reactivity profiles of CD13- and CD10-specific antibodies with human leukemic cells rarely overlap, mirroring the lineage-specific binding pattern of these reagents to normal hematopoietic cells. CD13 is expressed on the leukemic blasts from the majority of patients with myeloid leukemia (1–10), whereas CD10 is expressed on lymphoid leukemias with the phenotype of early B cell progenitors (62–65). The identification of these differentiation antigens as membrane-bound metalloproteases suggests new avenues for testing their physiologic roles on myeloid and lymphoid progenitors, and for determining their possible influence on normal and malignant hematopoietic cell development.

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Note added in proof. The CD13/aminopeptidase N cDNA sequence has been submitted to Gen Bank, accession number M22324.

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