

The B Cell Antigen CD75 Is a Cell Surface Sialyltransferase

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Summary

In this work we have isolated a cDNA clone encoding the B cell antigen CD75. The amino acid sequence of CD75 is shown to be identical to that of human α 2,6 sialyltransferase, believed to be primarily associated with the Golgi complex. This is the first demonstration of cell surface expression of sialyltransferase which, in B cells, may play an important role in intercellular adhesion and antigen presentation events.

CD75 has recently been characterized as a B cell surface molecule expressed predominantly on mature and activated B lymphocytes. The anti-CD75 mAb HH2 (1) has been found to augment DNA synthesis in B cells stimulated by anti-IgM antibodies. However, in some B cells lines, HH2 mAb produced an inhibitory effect upon DNA synthesis, suggesting that CD75 may be involved in regulating B cell activation. In this work, we show that a cDNA clone encoding CD75 is identical to a human sialyltransferase (2, 3) and 80% homologous to the rat α 2,6 sialyltransferase (4). Sialyltransferases catalyze the attachment of sialic acids to the termini of oligosaccharides and are typically located in the Golgi complex. Cell surface expression of a sialyl transferase in B cells is particularly interesting in light of the observations that sialation of cell surface oligosaccharides plays a role in regulating a variety of cellular functions including cell-cell interaction (5), antigen presentation (6), and activation (7).

Materials and Methods

Isolation of the CD75 cDNA Clone. A cDNA library derived from the Burkitt lymphoma Daudi cell line (8) was introduced into COS cells by the DEAE-Dextran method (9, 10) and screened with the HH2 mAb by successive rounds of panning and enrichment as described (9, 10).

Sequencing, RNA Hybridization. The CD75 cDNA clone was subjected to digestion with several restriction endonucleases and subcloned into Bluescript SK⁻ and KS⁻ vectors. Recombinant clones were sequenced by the dideoxy method, modified to allow the use of fluorescein-labeled primers (11). Sequencing products were analyzed by an automated sequencer (EMBL, Heidelberg, FRG) (12, 13). T7 DNA polymerase, dNTPs, and ddNTPs were purchased from Pharmacia-LKB (Bromma, Sweden).

Total cellular RNA was isolated by the LiCl/urea method (14), loaded onto a 1% agarose gel, electrophoresed, transferred to nylon

membranes, and hybridized to ³²P-labeled CD75 cDNA as described (15).

Results and Discussion

After three rounds of selection, one of four randomly picked colonies reacted with mAb HH2 by indirect immunofluorescence. The cDNA clone was reintroduced into COS cells and found to react with all of the CD75 mAb (16).

RNA blot analysis revealed a major 4.7-kb message expressed to a variable degree in three Burkitt lymphoma cell lines Daudi, Raji, and Reh and very weakly in the T cell leukemia Jurkat (Fig. 1). An identical message was expressed in peripheral blood B cells, but only a very faint message was observed in normal resting T cells (Fig. 1).

Nucleotide sequence analysis (Fig. 2) showed that the cDNA insert consisted of 1,540 bp with a single open reading frame of 1,215 bp encoding a 405 residue polypeptide with predicted *M_r* 46,367. The first ATG is embedded in an atypical initiation codon sequence, although an A and a C are found at positions -3 and -4 respectively, consistent with the consensus initiation codon sequence (17). Absence of a consensus leader peptide (18) suggests that the NH₂-terminal domain is intracellular. The first 9 residues are followed by a stretch of 18 predominantly hydrophobic amino acids consistent with a transmembrane domain. The extracellular domain is predicted to consist of 378 residues and contain two potential N-linked (Asn-Xaa-Ser/Thr, where Xaa cannot be a Pro) glycosylation sites. Comparison with other known sequences using the NBRF data base showed that the predicted peptide sequence is identical to that encoded by cDNA clones specific for a human sialyltransferase (2,3 EMBL accession No. X17247), with three exceptions: the substitution of a lysine for a leucine residue at position 27; the substitution of a threo-

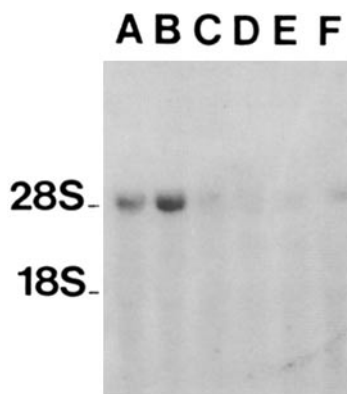


Figure 1. RNA blot analysis of human B and T cells and cell lines hybridized to CD75 cDNA probes. (Lane A) Burkitt lymphoma Daudi; (lane B) Burkitt lymphoma Raji; (lane C) Burkitt lymphoma Reh; (lane D) T cell leukemia Jurkat; (lane E) normal resting T cells; (lane F) peripheral blood B cells.

nine for an arginine residue at position 73; and the absence of a histidine residue at position 72 rendering the CD75 sequence one residue shorter than the predicted sialyltransferase sequence (3). Comparison of CD75 with rat $\alpha 2,6$ sialyltransferase (4) showed 80% homology. The region of greatest divergence between rat and human sequences occurs between residues 34 and 106, where the homology is only 50%. No homologies with other known proteins were found.

Sialyl transferases belong to the broad family of glycosyltransferases, a group of integral membrane proteins of the endoplasmic reticulum and Golgi apparatus involved in the

synthesis of glycoprotein and glycolipid sugar chains (19). Although all of the cloned glycosyltransferase genes encode type II integral membrane proteins, the amino acid sequences bear little homology to each other (19). However, interspecies homology for each enzyme is highly significant (19).

β -Galactoside $\alpha 2,6$ sialyltransferase, like other members of the sialyltransferase family, catalyzes the transfer of sialic acid to the terminal positions of the carbohydrate groups of glycoproteins in the general reaction: CMP-SA + HO acceptor-CMP + SA-O-acceptor (4). The principal localization of sialyltransferases is believed to be the Golgi apparatus where they participate in post-translational glycosylation pathways. The present work suggests that an additional major site of sialyltransferase expression, at least in B cells, is the cell surface, which may have several important functional implications.

Sialic acid is an important constituent of many cell surface proteins and is believed to help conceal surface structures in certain circumstances (20). In tumors, sialation of histocompatibility antigens may provide a shield against recognition by cytolytic T cells (7, 21). In physiologic situations, sialic acid may regulate homotypic adhesion of N-CAM (22) and antigen presentation by accessory cells (6).

B cells are highly motile and communicate with a variety of other cell types as well as components of the extracellular matrix. Activation induces modifications in the B cell phenotype, particularly with respect to cell surface expression of molecules involved in cell-cell adhesion and antigen presentation. By virtue of its capability to modify cell surface molecules, the sialyltransferase CD75 may play an important role in several sectors of B cell biology and provide a possible mechanism for B cell malignancies to elude immune surveillance.

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1  TGGAAAGCTCTGCATCCCTTCTCCCATACCTTGCTCTACACATCTCTTCATGTGTATCCTCTGCAGCATCCTTGATGATAAACCAGTAAATATGACTTTTGATCATCTGAGAAAATGGGC
121 CTTGGCCTGCAGACCCAATAAACTTCTCGAATGGATAATAGTGCAAAATCCTGTGGACCTGAAGGGCCTGCCGCCCTGTGGGGGATTAGCCAGAAGCAGCGTTGTTTCCCGCTCAG
241 AACAAAGTGACTTCCCTGAACACATCTTCATTATGATTACACCAACCTGAAGAAAAAGTTGAGCTGCTGCCGCTCCTGGCTTCTCTCTGTTGAGTCATCTGTGTGGTGGAAAAGA
1  M I H T N L K K K F S C C V L V F L L F A V I C V W L E K K
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361 AGAAAGGGAGTTACTATGATTCCTTTAAATGGCAAACCAAGGAATCCAGGTGTTAAAGAGTCTGGGGAAATGGCCATGGGGTCTGATTCCAGTCTGTATCCTCAAGCAGCACCCAGG
31  K G S Y Y D S F K L Q T K E F Q V L K S L G K L A M G S D S Q S V S S S S T Q D
-----
481 ACCCCACAGGCCCGCAGACCCCTCGGAGTCTCAGAGCCCTAGCCAAGGCCAACCAGAGGCCCTCCTCCAGGTGTTGAACAAAGGACAGCTCTTCCAAAACCTTATCCCTAGGCTGCAAA
71  P T G R Q T L G S L R G L A K A K P E A S P Q V W N K D S S S K N L I P R L Q K
-----
601 AGATCTGGAAGAATTACCTAAGCATGAACAAGTACAAGTGTCTACAAGGGGGCAGGACCAGGCATCAAGTTCAAGTGCAGAGGCCCTGCCCTGCCACCCCTGGGACCATGTGAATGTAT
111 I W K N Y L S M N K Y K V S Y K G P G P G I K F S A E A L R C H L R D H V N V S
-----CHO-----
721 CCATGGTAGAGGTCACAGATTTTCCCTTCAATACCTCTGAATGGGAGGTTATCTGCCCAAGGAGAGCATTAGGACCAAGGCTGGGCCCTTGGGGCAGGTGTGTGTTGTGTGTCGTCAGCGG
151 M V E V T D F P F N T S E W E G Y L P K E S I R T K A G P W G R C A V V S S A G
---CHO---
841 GATCTCTGAAGTCTCCCACTAGGACAGAGAAATCGATGATCATGACGAGTCTGAGGTTTAAATGGGGACCCACAGCCAACCTCCAACAAGATGTGGGCACAAAACCTACCATTCGGC
191 S L K S S Q L G R E I D D H D A V L R F N G A P T A N F Q Q D V G T K T T I R L
-----
961 TGATGAACCTCAGTTGGTTACCACAGAGAAGCGCTTCCCAAAAGACAGTTTGTACAATGAAGAACTCTAATGTATGGGACCCATCTGTATACCACCTCAGATATCCCAAAGTGGTACC
231 M N S Q L V T T E K R F L K D S L I Y N E G I L I V W D P S V Y E H S D I P K W Y Q
-----
1081 AGAATCCGGATTATAATTTCTTTAACTACAAGACTTATCGTAAGCTGCACCCCAATCAGCCCTTTTACATCTCAAGCCCCAGATGCCTTGGGAGCTATGGGACATCTTCAAGAAA
271 N P D Y N F F N N Y K T Y R K L H P N Q P F Y I L K P Q M P W E L W D I L Q E I
-----
1201 TCTCCCAGAGAGATTACGCCAAACCCCACTCCTGCGGATGCTTGGTATCATCATGATGACGCTGTGTGACCAGGTGGATATTTATGAGTTCTCCCATCAAGCCGCAAGACTG
311 S P E E I Q L V T T S M N P P S S G M L G I I I M M T L C D Q V D I Y E F L P S K R K T D
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1321 ACGTGTGCTACTACTACAGAGTTCTTCGATGTGCTGACAGATGGGTGCTTACCACCCGCTGCTCTATGAGAAGAAATTTGGTGAAGCATCTCAACCAGGGCAGACATGAGGACATCT
351 V C Y Y Y Q K F F D S A C T M G A Y H P L L Y E K N L V K H L N Q G T D E D I Y
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1441 ACCTGCTTGGAAAAGCCACACTGCCTGGCTTCCGGACCATCACTGCTAAGCACAGGCTCCTCACTTCTCCATCAGGCATTAATGAATGGTCTCTTTG
391 L L G K A T L P G F R T I H C *

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Figure 2. Nucleotide and deduced amino acid sequence of CD75. The predicted transmembrane domain is underscored. Potential sites of N-linked glycosylation are indicated -CHO-.

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