

Cloning of a Carcinoembryonic Antigen Gene Family Member Expressed in Leukocytes of Chronic Myeloid Leukemia Patients and Bone Marrow¹

Barbara Berling, Frank Kolbinger, Fritz Grunert, John A. Thompson, Frank Brombacher, Franz Buchegger, Sabine von Kleist, and Wolfgang Zimmermann²

Institut für Immunbiologie, Universität Freiburg, Stefan-Meier-Strasse 8, D-7800 Freiburg, Federal Republic of Germany [B. B., F. K., F. G., J. A. T., F. Br.]; Max Planck Institut für Immunbiologie, Stübweg 51, D-7800 Freiburg, Federal Republic of Germany [F. B.]; and Institut de Biochimie, Université de Lausanne, Chemin des Boveresses 155, CH-1066 Epalinges, Switzerland [S. v. K., W. Z.]

ABSTRACT

The carcinoembryonic antigen (CEA) gene family belongs to the immunoglobulin superfamily and can be subdivided into the CEA and pregnancy-specific glycoprotein subgroups. The basic structure of the encoded proteins consists of, in addition to a leader, one IgV-like and 2, 3, or 6 IgC-like domains. These domains are followed by varying COOH-terminal regions responsible for secretion, transmembrane anchoring, or insertion into the membrane by a glycosyl phosphatidylinositol tail. Here we report on the characterization of *CGM6*, a new member of the CEA gene subgroup, by complementary DNA cloning. The deduced coding region comprises 349 amino acids and consists of a leader, one IgV-like, two IgC-like domains, and a hydrophobic region, which is replaced by a glycosyl phosphatidylinositol moiety in the mature protein. *CGM6* transcripts were only found thus far in leukocytes of chronic myeloid leukemia patients, in normal bone marrow, and in marginal amounts in normal granulocytes. The *CGM6* gene product might, therefore, represent a myeloid marker. Analyses of *CGM6* protein-expressing HeLa transfectants with monoclonal antibodies strongly indicate that the *CGM6* gene codes for the CEA family member NCA-95.

INTRODUCTION

CEA³ is a marker in human tumors of epithelial origin (1). Although it lacks absolute tumor specificity, it is widely used for the postoperative surveillance of intestinal cancer patients (1). Earlier immunological studies identified a number of closely related molecules which cross-react with CEA (reviewed in Ref. 2). Recent findings suggest that CEA and related antigens might function as cell adhesion molecules and/or ecto-ATPases (3-5). The CEA-related proteins are all highly glycosylated and show a large degree of variability with respect to their molecular sizes and expression patterns. For example, CEA and two glycosylation variants encoded by the closely related *NCA* gene (*NCA-50* and *NCA-90* or *TEX*) have been described to be present in colon adenocarcinomas and in smaller amounts in normal colonic mucosa (6-8), whereas in normal granulocytes, *NCA-90*, *NCA-95*, and *NCA-160* are expressed (9, 10). The CEA-related proteins are encoded by a gene family (reviewed in Ref. 11). Analysis of the genes, as far as they are known, indicate a basic domain structure for these molecules as well as homology to the immunoglobulin supergene family. The proteins consist of a leader peptide, an NH₂-terminal or IgV-like domain, a varying number of IgC-like domains, and a short

COOH-terminal domain, which may or may not allow membrane anchorage. In the biliary glycoprotein 1, another member of the CEA family, the IgC-like domains are followed by a transmembrane and a cytoplasmic domain (12-14). For CEA and the *NCA-50/90*, a short hydrophobic COOH-terminal region is posttranslationally replaced by a GPI moiety (15-18). Recently, a second major subgroup has been identified as belonging to the CEA family based on sequence comparisons. These molecules represent the heterogeneous PSG, for which about 10 genes have so far been defined (reviewed in Ref. 19).

In a previous report from our group on the expression of CEA-related genes, tissue-specific expression patterns for the CEA and the *NCA* gene could be determined (20). These studies also revealed the existence of a CEA-related, 2.2-kilobase mRNA species in CML leukocytes, which was not found in the other tissues investigated. This finding indicates that this CEA-related mRNA or its encoded protein may be of potential use either as a tumor or a lineage marker. For this reason, it is important to characterize this mRNA more closely. In this presentation, we report on the isolation of corresponding cDNA clones and detailed analyses regarding structure and expression, as well as tentative assignment to the *NCA-95* protein.

MATERIALS AND METHODS

Monoclonal Antibodies. The mouse monoclonal antibodies 26/3/13, 4/3/17, 47, 192, and 202 were raised against purified CEA and N1 against *NCA-50* (6, 21). Their specificities have been described before (6, 9, 10, 22, 23).

Cells and Tissues. HeLa cells were cultured at 37°C in Falcon (Becton Dickinson, Heidelberg, FRG) dishes containing RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. Human primary tumors or human tumors propagated in nude mice (24) were frozen and stored in liquid nitrogen immediately after resection. Leukocytes from CML patients as well as granulocytes and lymphocytes from healthy adults were isolated from peripheral blood or buffy coats, respectively, by centrifugation through a Percoll (Pharmacia, Freiburg, FRG) step gradient according to the manufacturer's protocol and were directly used for RNA isolation. Bone marrow was obtained from a healthy donor and used without further fractionation.

Isolation and Characterization of cDNA Clones. Phage DNAs of a λ -gt11 cDNA library constructed from the spleen of a CML patient (Clontech, Palo Alto, CA) were hybridized on nitrocellulose filters in replicas with the ³²P-labeled (25) DNA fragment covering the coding region of an *NCA* cDNA clone (26) in 6× SSPE (1× = 0.18 M NaCl-10 mM sodium phosphate, pH 7.4-1 mM EDTA)-5× Denhardt's solution [1× = 0.02% each of Ficoll (Pharmacia, Freiburg, FRG), bovine serum albumin fraction V (Serva, Heidelberg, FRG), polyvinyl pyrrolidone], 0.5% SDS, 30% formamide at 42°C overnight. The filters were washed in 2× SSPE, 0.1% SDS at 60°C for 1 h. The positive clones were plaque purified three times (27). The *EcoRI* cDNA inserts were subcloned into M13 vectors (Boehringer Mannheim, Mannheim, FRG) and sequenced according to the method of Sanger *et al.* (28) with the Sequenase sequencing kit (United States Biochemicals: Renner, Dann-

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² To whom requests for reprints should be addressed.

³ The abbreviations used are: CEA, carcinoembryonic antigen; *NCA*, nonspecific cross-reacting antigen; CML, chronic myeloid leukemia; GPI, glycosyl phosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C; IgV, immunoglobulin variable; IgC, immunoglobulin constant; PSG, pregnancy-specific β_1 -glycoprotein; CML, chronic myeloid leukemia; cDNA, complementary DNA; FRG, Federal Republic of Germany; SSPE, sodium-salt-phosphate-EDTA; SDS, sodium dodecyl sulfate; MAb, monoclonal antibody.

stadt, FRG) using universal and 17mer internal oligonucleotide primers. For comparison of the nucleotide and amino acid sequences the computer program "Align" was used.

RNA Purification and Northern and Southern Blot Hybridizations. Total RNA was extracted from fresh cells or from tissues pulverized in liquid nitrogen by the guanidinium thiocyanate method (29) or by the acid phenol method (30). For Northern blot hybridizations, RNAs were electrophoresed on 1% agarose gels containing 10 mM methylmercury hydroxide, transferred to charged nylon membranes (GeneScreen Plus, Du Pont de Nemours, Dreieich, FRG), and hybridized with ³²P-labeled cDNA fragments as described before (20). The cDNA fragments were labeled either by priming with random hexamers (25) or with the universal KS primer on a *Bgl*I fragment from the Bluescript plasmid (Stratagene, Heidelberg, FRG) with the subcloned CGM6a cDNA, which contains the 3'-untranslated region of CGM6 cDNA (Fig. 1A). The blots were washed under low (2× SSPE, 1% SDS, 60°C) and high stringency conditions (0.1× SSPE, 0.1% SDS, 62°C) for 45 min, respectively. Southern blot hybridizations were performed as described before (20), apart from the final wash, which was at 62°C.

Construction of pBEH/CGM6 Expression Plasmid. First, a cDNA clone with the complete open reading frame of CGM6 was constructed in the Bluescript vector using the unique *Tth*1111 site, present in both λ-CGM6a and λ-CGM6b cDNAs (Fig. 1A). The resulting full-length cDNA fragment was excised with the restriction endonucleases *Xma*I and *Hind*III, which both cut in the flanking polylinker regions, and cloned into the *Xma*I/*Hind*III sites of the expression vector pBEHpUC9 (31) downstream of the SV40 early promoter. The identity of the constructs was confirmed by restriction endonuclease and partial sequence analysis.

Stable Transfection and Identification of CGM6-expressing HeLa Cells. Transfection of HeLa cells was performed by the method of Felgner *et al.* (32) as described recently (33). For cotransfection, 1 × 10⁶ HeLa cells on a 10-mm plastic Petri dish were treated with 1 μg pSV2neo (34) which confers resistance toward the neomycin-derivative G418, a 20-fold molar excess (20 μg) of the CGM6 expression construct pBEH/CGM6, and 40 μg of Lipofectin (GIBCO-BRL, Karlsruhe, FRG) in 3 ml RPMI 1640 without serum. Control cultures were transfected with 1 μg of pSV2neo DNA alone. Selection for cells expressing the bacterial *neo* gene was carried out in the presence of 0.6 mg/ml of the active antibiotic G418 (GIBCO-BRL). After 4 weeks colonies were isolated using cloning cylinders.

Flow Cytometry Analysis. Flow cytometry (35) of cloned G418-resistant cells was performed on a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA) as described before (33). The optimal concentrations of the monoclonal antibodies were determined by serial dilutions.

PI-PLC Treatment. Adherent cells were removed from culture dishes with phosphate-buffered saline (without Ca²⁺ and M²⁺ ions) containing 0.5 mM EDTA and singled by pipeting. Cells (1 × 10⁶) in 200 μl phosphate-buffered saline, 3% fetal calf serum, 0.1% sodium azide were incubated with 0.6 unit/ml PI-PLC from *Bacillus thuringiensis* (Dianova, Hamburg, FRG) for 3 h at 37°C. Cells were recovered by centrifugation for 5 min at 400 × *g* and analyzed by flow cytometry as described before (33).

RESULTS

Isolation and Analyses of CGM6 cDNA Clones. For the isolation of cDNA clones, which correspond to the 2.2-kilobase CEA-related mRNA species found in CML leukocytes (20, 36), we screened 1 × 10⁶ plaques of a λ-gt11 cDNA library prepared from the spleen of a CML patient. As a probe we used the coding region of the NCA cDNA clone 9 (26). Four positive clones were identified. Partial sequence analyses revealed that two of these clones are derived from NCA mRNA, which is also found in CML leukocytes (20). The inserts of the two

remaining clones were completely sequenced according to the strategy shown in Fig. 1A. Both cDNA fragments overlap and together cover 32 base pairs of the 5'-noncoding region, a 1047-base pair open reading frame, and 285 base pairs of the 3'-untranslated region (Fig. 1B). The encoded putative preprotein consists of 349 amino acids with a relative *M_r* of 38,125 (minus leader: *M_r* 34,326). Comparison of the deduced amino acid sequence with that of NCA (26) or PSGs (37) revealed that the putative CGM6 protein has a similar domain organization, consisting of a 34-amino acid leader peptide (L), an NH₂-terminal or IgV-like domain of 108 amino acids (N), two IgC-like domains (type A and B) of 92 and 86 amino acids, respectively, with the characteristic pairs of conserved cysteine residues, and a 29-amino acid hydrophobic membrane domain (M)

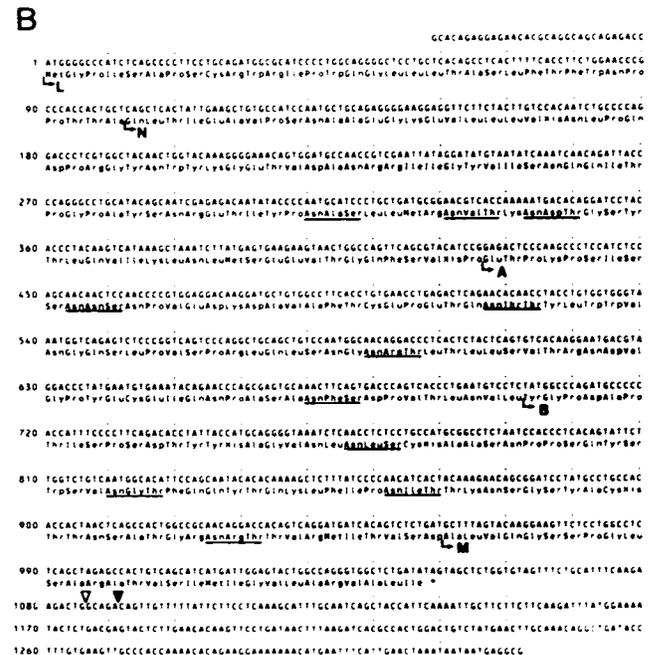
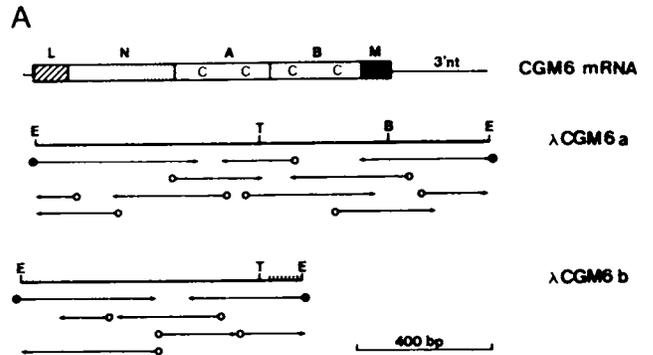


Fig. 1. Domain organization (A) and nucleotide sequence and deduced amino acid sequence (B) of the CGM6 cDNA. A: boxes, domains of the coding region of CGM6 mRNA (L, leader; N, NH₂-terminal or IgV-like domain; A, B, IgC-like domain type A and B; M, membrane domain; C, cysteine); thin lines, noncoding regions. CGM6a and CGM6b cDNAs were sequenced with internal (○) or universal (●) primers according to the strategy shown by arrows. Dotted line in CGM6b, an unspliced intron sequence. The *Tth*1111 (T) and *Bgl*II (B) restriction endonuclease sites used for construction of the full-length cDNA and preparation of the hybridization probe, respectively, are shown. The flanking *Eco*RI (E) sites have been created by the cloning procedure. In B, the nucleotide sequence is numbered from the start codon. Arrows, domain borders; underline, consensus sequences for NH₂-glycosylation; open arrowhead, end of the membrane domain exon in the CEA gene; closed arrowhead, point of divergence to the sequence of NCA cDNA.

* M. Trippel and R. Friedrich, unpublished data.

(Fig. 1). Eleven potential sites for NH₂-glycosylation are present, all of them at conserved positions in comparison with the NCA-50/90 protein, which has one additional glycosylation site (26).

The nucleotide sequences of λ -CGM6a and λ -CGM6b diverge directly downstream of the A domain (Fig. 1A). Comparison with intron sequences of the *NCA* gene⁵ revealed that the clone λ -CGM6b is obviously derived from an incompletely spliced mRNA precursor, since it contains an intron sequence between the exons encoding the A and B domains as found for other thus far characterized members of the *CEA* gene family (11, 38, 39).

Furthermore, comparison of the nucleotide sequence of the various domains of CGM6 with the corresponding sequences of members of the CEA and PSG subgroups clearly indicate that CGM6 belongs to the CEA subgroup. The highest overall similarity for the coding region is found with NCA (85% at the nucleotide and 78% at the amino acid level), which has the same domain organization as CGM6 (L-N-A-B-M). The sequence similarity with NCA mRNA, however, stops abruptly 41 nucleotides beyond the CGM6 stop codon. This position corresponds closely to the site of divergence of the CEA and NCA mRNA (26). Beyond this position a rather high similarity (75%) to the corresponding sequence of CEA mRNA is found (Fig. 1B). However, CGM6 mRNA lacks the Alu-type repetitive element, which is inserted in CEA mRNA 120 nucleotides downstream of the stop codon (40).

CGM6 mRNA Is Specifically Expressed in CML Leukocytes and in Bone Marrow. To prove that the CGM6 clones are derived from the 2.2-kilobase mRNA present in CML leukocytes, Northern blot analyses with total RNA from pooled leukocytes of several CML patients were performed. With a probe from the coding region of NCA, which hybridizes under the applied conditions with the mRNAs of all members of the CEA subgroup, two mRNA species of 2.2 and 2.5 kilobases could be detected (Fig. 2A, lane 2). As a probe for CGM6 mRNA a DNA fragment covering the 3'-untranslated region of λ -CGM6a was used. Under stringent conditions, the 2.2-kilobase mRNA and to a lesser degree a 3.0-kilobase RNA species were found to hybridize, whereas a probe specific for NCA mRNA (20) hybridized with the 2.5-kilobase mRNA species (Fig. 2A, lanes 1 and 3). In leukocytes of four other individual CML patients, the 2.2- and 3.0-kilobase RNA species could also be detected in variable amounts with the CGM6 probe under stringent conditions. The Northern blot experiment for two of these CML RNAs are shown in Fig. 2B (lanes 1 and 2). An mRNA species of apparently the same size as the 2.2-kilobase species of CML leukocytes was also identified in total RNA from normal bone marrow along with three high molecular weight RNA species, not found in CML leukocytes (Fig. 2C, lane 2). Due to their large size, the latter RNAs could represent CGM6 mRNA precursors. None or only marginal amounts of the 2.2-kilobase mRNA species were found in total RNA from lymphocytes and granulocytes, respectively, from healthy donors, although comparable amounts of β -actin mRNA were present in these RNA preparations (Fig. 2B). To further determine the expression pattern of CGM6-related mRNAs we have analyzed a number of human tumors and metastases as well as a few normal tissues by Northern blot hybridization. No CGM6 mRNA could be detected in any of the following tissues examined: one adenocarcinoma of the

pancreas, one of the lung and two of the breast, two of the stomach and five of the colon, three serous and four mucinous carcinomas of the ovary, one squamous carcinoma of the cervix and of the lung, one small cell and one large cell carcinoma of the lung, one thyroid gland, one gallbladder and one liver carcinoma, one kidney hypernephroma, one osteosarcoma and one pleural mesothelioma, and normal colonic mucosa and placenta, although in all cases a signal for β -actin mRNA was found (data not shown). The lack of a hybridization signal with RNA from colon tumors as well as from placenta, which contain CEA and PSG mRNAs, respectively (20, 37), rules out a cross-hybridization of the CGM6 probe with these mRNAs, which show in the corresponding regions 75 and 60% [PSG3 mRNA (41)] similarity, respectively (42). To examine, whether the 2.2-kilobase, the 3.0-kilobase and the high molecular weight RNA species are derived from multiple, closely related genes or from one gene, a Southern blot with *EcoRI*- or *SstI*-digested human genomic DNA was hybridized with the same CGM6 probe. One major and one or two minor hybridizing fragments could be observed under stringent conditions (Fig. 2D, lanes 1 and 2). The faint bands could have been caused by cross-hybridization with closely related genes or the probe used may contain information from more than one exon (see below). As expected from the Northern blot experiments, genomic DNA fragments of different sizes (*EcoRI*, 2.7 kilobases; *SstI*, 13 kilobases) hybridized with a probe from the related 3'-untranslated region of the CEA cDNA (Fig. 2D, lanes 3 and 4), which is in good agreement with those found by restriction mapping of the CEA gene (43).

The CGM6 Gene Probably Encodes NCA-95. We have attempted to resolve the question, whether the CGM6 gene encodes a known but not yet assigned member of the CEA family (2). For this purpose, we examined G418-resistant HeLa cell clones, which had been obtained by transfection with a CGM6 cDNA-containing expression vector together with a G418 resistance-conferring plasmid (pSV2neo) with MAbs of varying specificity (Table 1). As a control, HeLa cell clones which were transfected with pSV2neo alone was used. Mab 47, which recognizes CEA and NCA-95 (9), reacted with a CGM6 HeLa transfectant (HeLa-CGM6/1) but not with the HeLa-neo1 (Fig. 3A). On the other hand, Mab 192, which recognizes an epitope present on CEA, NCA-50/90, and NCA-160 but apparently not on NCA-95 (9, 10), stained HeLa-CGM6/1 cells only marginally (Fig. 3B). NCA-producing HeLa cells transfected with the plasmid pBHE/NCA (33) were strongly labeled with Mab 192 which was comparable to the reaction of HeLa-CGM6/1 cells with Mab 47 (data not shown). Therefore, the marginal reaction of Mab 192 with the CGM6-expressing cells is probably due to a weak cross-reaction of the CGM6-encoded protein not seen in immunoprecipitation experiments rather than a generally low affinity of Mab 192 to CEA-related molecules. This interpretation is substantiated by the observation that Mab 202, which binds to CEA, NCA-50, and NCA-95, also strongly reacted with HeLa-CGM6/1 cells (Fig. 3C). A marginal reaction of the HeLa-CGM6/1 transfectant was also observed with a Mab specific for the two glycosylation variants NCA-50 and NCA-90 (Mab N1) and with Mab 4/3/17 (Fig. 3, D and E), which recognizes both CEA and a M, 160,000 NCA species (33). These reactions are possibly due to the presence of small amounts of CEA-like antigen(s) endogenous to HeLa cells rather than to the expression of the CGM6 protein because the negative control (HeLa-neo1) was labeled to the same extent by these antibodies (Fig. 3, D and E). A Mab specific for CEA (MAB 26/3/13) did not react

⁵J. Thompson, unpublished results.

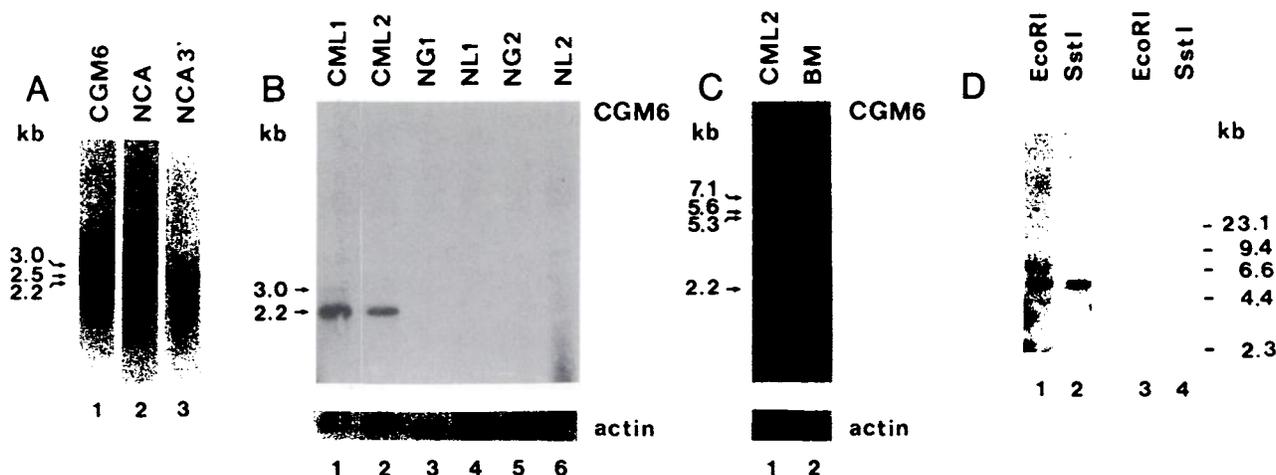


Fig. 2. Northern blot analyses of CGM6 transcripts in myeloid and lymphoid cells. Total RNA (10 μ g) from pooled leukocytes of six CML patients (A, lanes 1-3) or leukocytes of individual CML patients (B, lanes 1 and 2; C, lane 1) and from normal granulocytes (B, lanes 3 and 5), normal lymphocytes (B, lanes 4 and 6), or bone marrow (C, lane 2) of healthy donors were used. After electrophoretic separation of the RNAs in methylmercury hydroxide-containing agarose gels and transfer to charged nylon membranes, the RNAs were hybridized with a 32 P-labeled probe covering the 3'-untranslated region of the CGM6 cDNA (A, lane 1; B; and C). After decay of the probe used before, the blots were rehybridized with probes from the coding [the 500-base pair *Pst*I and the 500-base pair *Pst*I/*Eco*R1 fragment of clone 9 (26)] or 3'-untranslated region of NCA cDNA [the 1.45-kilobase *Eco*R1 fragment of clone 9 (26)], respectively (A, lanes 2 and 3), or a mouse β -actin probe [(49); B and C]. D, Southern blot analysis of the CGM6 and CEA genes. Human DNA (2.5 μ g) digested with *Eco*R1 and *Sst*I, respectively, were size fractionated, blotted, and hybridized with the probe from the 3'-untranslated region of the CGM6 cDNA (lanes 1 and 2). Subsequently, after stripping, this membrane was rehybridized with a 408-base pair *Rsa*I/*Pst*I fragment from the 3'-untranslated region of a CEA cDNA clone [(20); lanes 3 and 4]. All blots were washed under stringent conditions (0.1 \times SSPE, 62°C) except after hybridization with the probe from the coding region of the NCA cDNA or the β -actin probe (2 \times SSPE, 60°C).

Table 1 Reactivity of anti-CEA/NCA MAbs with purified antigens

The reactivity of the MAbs has been assessed by immunoprecipitation of the radioactively labeled CEA-related antigens (6, 9, 10, 22, 23). All MAbs were of the IgG1 isotype.

	CEA	NCA-50/90	NCA-95	NCA-160
MAb N1	-	+	-	-
MAb 47	+	-	+	-
MAb 4/3/17	+	-	-	+
MAb 26/3/13	+	-	-	+
MAb 192	+	+	-	-
MAb 202	+	+	+	ND ^a

^a ND, not determined.

with the CGM6 transfectant. (Fig. 3F).

The CGM6-encoded Protein Is Anchored to the Membrane by a GPI Moiety. In both CEA and NCA preproteins the hydrophobic membrane domain is replaced by a GPI anchor during maturation (15-18, 33). To investigate whether the homologous hydrophobic domain of the CGM6 protein is also substituted by a GPI tail and anchored to the membrane in the same

fashion, we have treated the CGM6 transfectant HeLa-CGM6/1 with bacterial PI-PLC, which specifically catalyzes the hydrolysis of the phosphate ester between the phosphatidyl and inositol moiety of the GPI anchor. Subsequent reaction with MAb 47 and flow cytometry revealed that most of the MAb 47 target was removed from the cells by PI-PLC treatment (Fig. 4).

DISCUSSION

In this paper we have described a new member of the CEA gene family, named CGM6. Altogether, six genes belonging to the CEA subgroup have been identified by analyses of genomic or cDNA clones: CEA, NCA, BGP, CGM1, CGM2, and CGM6 (19). The genes for CEA, NCA, and biliary glycoprotein 1 have been assigned to known members of the CEA protein family on the basis of partial amino acid sequences (13, 14, 18, 26, 40), whereas for the CGM1 and CGM2 genes no transcripts or

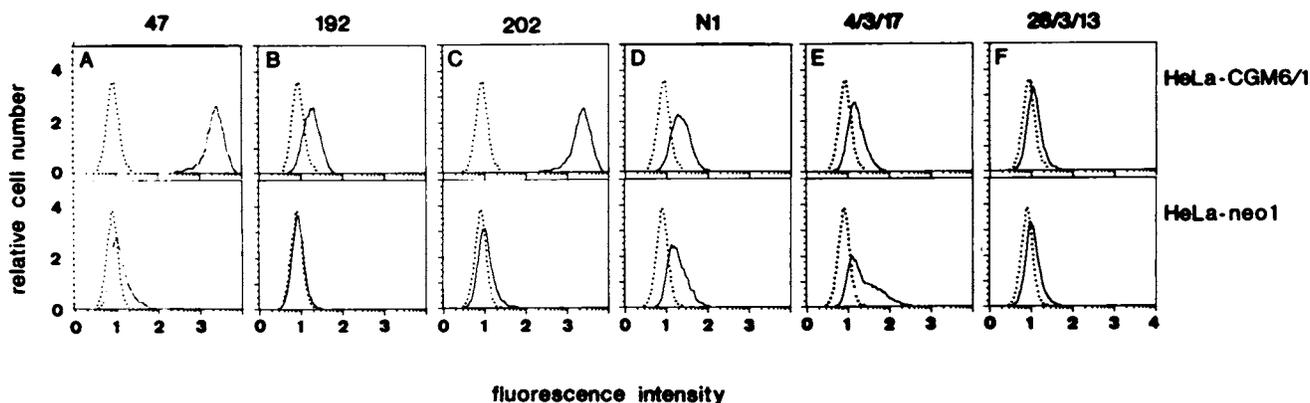


Fig. 3. Characterization of the CGM6 protein by MAbs. A HeLa cell line stably transfected with the CGM6 expression plasmid and pSV2neo (HeLa-CGM6/1) or a control cell line transfected with pSV2neo alone (HeLa-neo1) were reacted with various MAbs which recognize different CEA-related antigens and stained with biotinylated goat anti-mouse IgG1 and R-phycoerythrin-coupled streptavidin (—). For a negative control, the first antibody was omitted (·····). The degree of reaction of the antibodies was assessed by flow cytometric analysis of 10^4 cells each. The fluorescence intensity is shown in \log_{10} units.

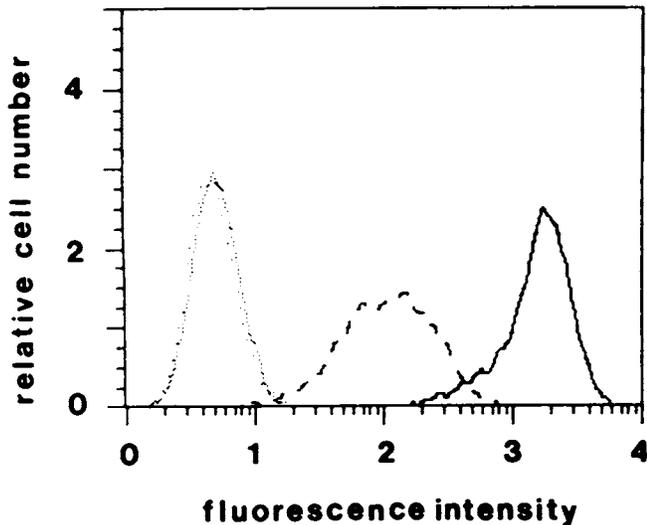


Fig. 4. Release of the CGM6 protein by PI-PLC-treatment. HeLa-CGM6/1 transfectants, before (—) and after treatment with PI-PLC (----) were stained with MAb 47 followed by biotinylated goat anti-mouse IgG1 and R-phycoerythrin-coupled streptavidin and analyzed by flow cytometry. As controls, cells with (.....) or without (....) PI-PLC treatment were analyzed omitting the primary antibody. The fluorescence intensity is shown in \log_{10} units.

proteins have been found so far (44).

In an attempt to determine the identity of the protein encoded by the CGM6 gene that is presumably expressed in CML leukocytes, it is important to know which CEA-related antigens are present in these cells. Three such antigens have been characterized in CML leukocytes⁶ and in normal granulocytes (9, 10). In addition to NCA-90, which is presumably identical with TEX (8) and has been shown by protein sequencing to be derived from the NCA gene (8), two other, not yet assigned NCA species of *M_r* 95,000 [formerly called NCA-97 by our group (6)] and 160,000 have been identified. These three antigens can be discriminated by a panel of monoclonal antibodies (33). The reaction pattern of a CGM6-expressing transfectant with MAb of different specificity strongly suggests that the CGM6 gene encodes NCA-95. For a definite assignment of CGM6 to NCA-95, however, partial amino acid sequences have to be obtained.

NCA-95 has been found in normal granulocytes (10), in CML leukocytes,⁶ in colonic tumors, and in normal lung tissue (6). The presence of NCA-95 in the latter two tissues is probably due to infiltrating granulocytes (9). This is in agreement with our results that CGM6-like mRNA species could only be detected in CML leukocytes and bone marrow cells but not in colon adenocarcinomas. Because CGM6 mRNA species could not be detected in a number of other tissues tested, CGM6 might represent a specific marker for the myeloid lineage, although more tissues still have to be tested. With polyclonal anti-NCA antibodies, which cross-react with all three above mentioned NCA species, myelocytes, metamyelocytes, segmented neutrophils, and partially promyelocytes, but not blast cells, were shown to express NCA(s) (45, 46). Further experiments have to be performed to identify the CGM6 mRNA-containing myeloid precursors. In normal mature granulocytes, CGM6 mRNA was only barely detectable. This could possibly be due to very low mRNA levels in these short-lived, terminally differentiated cells or to synthesis of this NCA species only occurring in granulocyte precursors, corresponding to the myeloid cell populations found in the peripheral blood of CML patients and in normal bone marrow.

⁶ Results to be published elsewhere.

Both CGM6 and NCA mRNA encode proteins with the same domain arrangement, including a hydrophobic domain, which is, however, five amino acids longer in CGM6. Despite this fact, this domain probably also serves as a signal for GPI tailing as in NCA and CEA (15–18, 33), as shown in this paper.

In the CEA gene this signal peptide, together with 39 nucleotides of the 3'-untranslated region, is encoded by a separate exon (43). Similarly, the transmembrane domain of BGP, which represents an extended CEA-like membrane domain, created by the insertion of a single nucleotide in front of the corresponding stop codon, is also encoded by a separate exon (12). Interestingly, in CEA, BGP, and NCA mRNA, the sequences diverge completely beyond the point which corresponds to the end of the CEA and BGP membrane exons. Therefore, it can be hypothesized that in the genes for CGM6 and NCA the 3'-noncoding regions are also disrupted by introns at the same positions. In analogy, the same exon/intron organization can be expected to exist for the *PSG2*, *PSG3*, and *PSG5* genes whose mRNAs have a CEA gene-related organization in their COOH-terminal domains and 3'-noncoding regions (37, 47).

The function of the CGM6 gene product (NCA-95) is unknown. Further studies are needed to clarify whether the CGM6 gene encodes a protein with a similar function as the product of the structurally closely related NCA gene, which has been shown to convey cell adhesion in transfected cells (4) or other recently described myeloid cell adhesion molecules (48). Furthermore, it will be interesting to know whether NCA-95 and NCA-90 perform their function on the same cells or on cells of different myeloid subpopulations.

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ADDENDUM

In parallel to our studies, Arakawa *et al.* (Biochem. Biophys. Res. Commun., 166: 1063–1071, 1990) characterized a cDNA clone (NCA-W272) from a normal WBC library, encoding a new species of NCA, which is identical to our CGM6 clones.

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