The Adhesion and Differentiation-inhibitory Activities of the Immunoglobulin Superfamily Member, Carcinoembryonic Antigen, Can Be Independently Blocked*

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Tissue architecture is established and maintained to a large extent by specific affinities of cell surface glycoproteins for molecules in the extracellular matrix or on the surface of adjacent cells. The latter are known collectively as cell adhesion molecules (CAMs)1 (1–3). CAMs function not only to fix cells in specific locations within tissues and regulate their movement but also to translate biochemical information from the extra-cellular environment through the activation of intracellular signaling pathways leading to specific functional cell responses (4, 5). CAMs are grouped into several different molecular families; the majority identified to date belong to the Ig superfamily (1, 6–8). Although the members of this family are functionally diverse, most are cell surface molecules involved in the recognition of other soluble or cell-associated molecules. All members of the Ig superfamily share conserved amino acid residues. These residues are limited to positions within the core of the Ig fold that are important for its structure. In contrast, the functional regions of the various members are often highly diverse.

Ig superfamily members function in many cases as a result of homophilic binding between their external domains or heterophilic binding interactions with other molecules (1, 9, 10). Homophilic interactions can be either antiparallel or parallel. Antiparallel interactions between molecules on apposed cell surfaces are required for intercellular binding (9, 11, 12). Parallel interactions between adjacent molecules on the same cell surface can facilitate this process by concentrating the binding molecules into synergistic arrays as described by the “Velcro” (13) or “zipper” models (14), in which the concerted action of multiple relatively weak interactions between individual pairs of molecules can lead to a strong overall bonding. Both types of interactions can also initiate signaling events (5, 14, 15). The clustering resulting from their combination might be expected to amplify these signals and lead to the triggering of threshold-activated signaling pathways (5, 14, 15).

The human carcinoembryonic antigen (CEA) family is of particular interest in terms of intermolecular binding because of the multiplicity and diversity of interactions between multiple closely related family members (13). We have investigated previously the structural requirements for CEA-mediated intercellular adhesion (9, 16). Intercellular adhesion seems to depend mainly on antiparallel CEA-CEA interactions as indicated by studies with hybrid constructs between CEA and neural cell adhesion molecule (9). CEA consists of a V-like Ig amino-terminal domain and three pairs of I-like Ig domains (denoted AXBX) that are terminated by a hydrophobic domain, which is processed to allow the addition of a glycophosphatidylinositol membrane anchor (17–19). Intercellular adhesion was

1 The abbreviations used are: CAM, cell adhesion molecule; CEA, carcinoembryonic antigen; DM, differentiation medium; GM, growth medium; mAb, monoclonal antibody; FACS, fluorescence-activated cell sorter; PBSF, phosphate-buffered saline plus 2% fetal bovine serum.

Received for publication, December 9, 2002, and in revised form, January 22, 2003.

Published, JBC Papers in Press, February 5, 2003, DOI 10.1074/jbc.M212500200
shown to be mediated by double reciprocal bonds between the N and A3B3 domains of antiparallel CEA molecules on apposed cell surfaces (9). Subdomains consisting of 5–6 amino acids that are required for intercellular adhesion, presumably as points of initial binding, were identified by mutational analysis of the amino-terminal domain (16).

Two cellular functions, intercellular adhesion (for review, see Stanners and Fukuyama (13)) and the inhibition of cell differentiation (20) have been shown to be dependent on homophilic CEA family member interactions. In particular, CEA was shown to block the myogenic differentiation of rat L6 myoblasts (20) and the neurogenic differentiation of mouse P19 embryonal carcinoma cells. The effect of CEA on differentiation would be expected to promote tumorigenic behavior and, in fact, was found to markedly increase the tumorigenicity of L6 myoblasts (21) and Caco-2 cells (22). Dereglulated overexpression of CEA and the closely related CEA family member, CEACAM6 (formerly known as NCA), at levels closely approximating those found in many colorectal carcinomas, has been shown recently to block cellular polarization, disrupt tissue architecture, and block the differentiation of human colonocyte cell lines (22). Consistent with these results, the cell surface level of CEA, which was determined by FACScan analysis of highly purified epithelial colonocytes from colorectal carcinomas and normal colonic tissue, was found to be elevated in the tumor cells at levels that were inversely correlated with the degree of differentiation (23). In this study, we have focused on the effects of CEA on the differentiation of L6 myoblasts because of the relative ease of experimentation. Although the expression of CEA in this system is ectopic, our experience to date indicates that the results obtained with this model system are closely mimicked by more biologically relevant systems such as human colonocytes (22, 24).

Because CEA and/or CEACAM6 are overexpressed in more than 50% of human cancers (25), it would seem experimentally and medically important to devise a means of releasing the CEA-mediated differentiation block and to determine whether interference with this function can be made selective without affecting the intercellular adhesion function. We show here that although the structural requirements for the intercellular adhesion and the differentiation block functions overlap, they can be separated effectively in the case of rat L6 myogenic differentiation. Using this information, small cyclized peptides and monovalent Fab fragments of monoclonal antibodies have been designed that are capable of releasing the myogenic differentiation block.

**EXPERIMENTAL PROCEDURES**

**Materials**—Cyclized and linearly blocked oligopeptides were obtained (>95% purity) from Multiple Peptide Systems (San Diego, CA). Linearly blocked peptides were rendered more stable by acetylation of the amino terminus and amination of the carboxyl terminus. Cyclic peptides contained two cysteine residues joined by sulfide bonds at their termini. The peptides used were blocked linear NaC-LFGYSWYKGE-NH2, NaC-VDGQRQIYG-NH2, NaC-RQQDTGFYG-NH2, and NaC-NAEGKEV-NH2; and cyclized H-CGYSWYKC-OH, H-CGNRQIIC-OH, H-CGNQIIC-OH, H-CGNQDGC-OH, and H-VCTDEKQCY-OH, representing subdomains 1, 2, and 3 of control peptides, respectively. Sequences actually present in the amino-terminal domain of CEA for the cyclized peptides are underlined.

**Construction of CEA cDNA Mutants**—Wild type cDNA coding for CEA (17) was used as a template for all PCR-generated constructs. The recombinant PCR technique (26) was used to generate site-directed mutants as described previously (16).

**Cell Culture**—Rat L6 myoblasts were grown as monolayer cultures at 37 °C in a humidified atmosphere with 5% CO2 in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (growth medium; GM). Cells were cultured under subconfluent to avoid the selection of non-fusing variants. LR-73 cells (27) derived from the Chinese hamster ovary line were grown in monolayer culture in a minimum essential medium (28) containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere with 5% CO2.

**Transfection**—L6 myoblast cells were seeded at 2 × 105 cells/100-mm plastic tissue culture Petri dish and cotransfected 24 h later by the calcium phosphate-mediated co-precipitation method as described previously (29), with 5 μg of pP1023B expression vector containing CEA (wild type or mutant) cDNA, 10 μg of LR-73 carrier genomic DNA, and 0.5 μg pSV2neo plasmid/dish. Stable pooled transfectant colonies were picked by selection with 1 mg/ml Geneticin (G418, Invitrogen). Immunofluorescent labeling with anti-CEA monoclonal antibody J22 (30) and FACS sorting were carried out to select for populations of transfectants stably expressing desired levels of mutant or wild type CEA on the cell surface. At least two independent pooled populations of transfectant clones were isolated for each transfected cDNA. All transfectant populations were maintained in GM containing 400 μg/ml G418. G418 was removed from the medium 24 h before each functional assay was performed.

**FACS Analysis**—Cells were removed from culture vessels by light trypsinization (a treatment that does not affect cell surface levels of CEA) and resuspended in ice-cold phosphate-buffered saline plus 2% fetal bovine serum (PBSF). 2 × 105 cells were incubated with polyclonal rabbit or monoclonal anti-CEA antibodies (J22 (30)) at a dilution of 1:100 in PBSF for 35 min on ice. Cells were washed with 2.5 ml of PBSF, centrifuged, and resuspended in 0.5 ml of PBSF containing fluorescein isothiocyanate-conjugated goat anti-rabbit antibody or anti-mouse antibody at a dilution of 1:100. After 30 min of incubation on ice, cells were washed, centrifuged, resuspended in 0.75 ml of PBSF, and analyzed using a BD Biosciences FACSScan instrument.

**Adhesion Assays**—LR-73 cells were seeded at 1 × 105/80-cm2 culture flask (Nalge Nunc Inc., Naperville, IL) in LR-73 growth medium. After 2 days in monolayer culture, the cultures were rendered as single cell suspensions by 3 min of incubation at 37 °C with 0.12% Bacto trypsin in phosphate-buffered saline lacking Mg2+ and Ca2+ and containing 15 mM sodium citrate. The cells were incubated at 105 cells/ml in α minimum essential medium containing 0.8% fetal bovine serum and 10 μg/ml DNase I at 37 °C with stirring at 100 rpm (29). The percentage of cells remaining as single cells, which declines over time because of formation of aggregates, was determined as a function of time by visual counting using a hemocytometer.

**Differentiation Assays**—To initiate fusion and differentiation, L6 cultures were seeded at 105 cells/cm2 at day 0 in 60- or 35-mm tissue culture Petri dishes of 7 × 105 cells/cm2 in multiwell plastic chamber slides (Nalge Nunc Inc.) and grown in GM. The medium was replaced after 3 days with Dulbecco’s modified Eagle’s medium plus 2% horse serum. Differentiation medium (DM), and the cells were fed an additional 5–7 days. To co-culture L6 (CEA) and L6 (ΔCEA) cell transfectants, cells were seeded at 3 × 105 cells of each type per 35-mm plastic tissue culture Petri dish in GM. 24 h later the medium was replaced with DM. For fusion index determinations, cells were fixed with 2.5% glutaraldehyde and stained with hematoxylin. The fusion index was calculated as the percentage of total nuclei contained in fused myotubes having more than three nuclei/myotube, as described previously (20). Fusion determinations were repeated three times (independent experiments) for each of two independently obtained transfectant populations for each mutant. The values reported in Figs. 5–7 represent the averages of these determinations.

**Cell Index**—As a biochemical measure of myogenic differentiation, cells were fixed in methanol:aceton (3:7) at −20 °C and processed for immunofluorescent staining with anti-myosin mAb (31).

To study the effect of cyclized or linearly blocked peptides on the CEA-mediated L6 differentiation block, cells were seeded at 7 × 105 cells/cm2 in 8-well chamber slides (Nalge Nunc Inc.) in GM on day 0. At 72 h of incubation, the medium was replaced with DM-containing peptide at the indicated concentrations.

**Purification of Antibodies**—Rabbit polyclonal anti-CEA antibody and mouse monoclonal anti-CEA antibodies (A20, B18, and D14) (30) were purified with the Bio-Rad Affi-Gel protein A MAPS II kit. Antibodies were added to differentiation medium to a final concentration of 1 mg/ml. Fab fragments of monoclonal anti-CEA antibodies were prepared as described previously (30). Fab fragments were added to differentiation medium to a final concentration of 100 μg/ml. The values for fusion indices shown in Fig. 10 represent the averages of three independent determinations.
Experimental Procedures.

NCEA (CEA) cells were co-cultured with an equal number of L6 (ΔNCEA) cells in GM, and 24 h later the medium was replaced with DM. After 5–7 days of incubation, fusion indices were measured as described under “Experimental Procedures.” Values shown represent the mean and standard error of measurements from three independent experiments.

RESULTS

Nature of CEA Homophilic Intermolecular Interactions Required for Differentiation Block—To explore the nature of the intermolecular interactions involved in the CEA-mediated block of the myogenic differentiation of L6 myoblasts (20), experiments were carried out to distinguish the requirements for parallel versus antiparallel intermolecular binding. For antiparallel interactions, non-differentiating stable L6 (CEA) transfectants were co-cultured with differentiation competent L6 (ΔNCEA) transfectants. ΔNCEA is a CEA cDNA deletion mutant lacking two-thirds of the amino-terminal domain (from amino acid 32 to 106) and is completely defective in mediating both intercellular adhesion (9) and the differentiation block of L6 myoblasts (20). CEA-expressing cells can bind to ΔNCEA-expressing cells, consistent with the model that the amino-terminal domain (intact in CEA) on a CEA-expressing cell can bind to the A3B3 domain (intact in ΔNCEA) on a juxtaposed ΔNCEA-expressing cell by an antiparallel mechanism (9). If such antiparallel interactions are sufficient for the differentiation blocking function of CEA, the CEA-expressing myoblasts should be able to “trans-inhibit” the differentiation of the ΔNCEA-expressing myoblasts. Co-culturing CEA-expressing L6 cells with an equal number of ΔNCEA-expressing L6 cells inhibited the overall differentiation (assessed by fusion into multinucleated myotubes) to a significantly lower level than co-culturing the same CEA-expressing cells with an equal number of non-CEA-expressing parental L6 cells for which trans-binding mediated by CEA would be impossible (Fig. 1). The latter co-culture controls for the dilution of differentiating cells with non-differentiating L6 (CEA) cells. This experiment therefore supports the contention that antiparallel CEA-CEA interactions between cells are sufficient for the CEA-imposed myogenic differentiation block.

To test for the role of parallel CEA-CEA interactions on the same cell surface, differentiation-competent L6 (ΔNCEA) transfectants were treated with cross-linking polyclonal and monoclonal anti-CEA antibodies. Antibodies for which the binding epitopes are still intact in the ΔNCEA molecule, rabbit polyclonal and D14 (binding epitope at the B2-A3 junction (30)), converted ΔNCEA to a differentiation blocking molecule, whereas control antibodies directed to binding epitopes that are missing in ΔNCEA, A20 and B18, two N domain-specific mAbs (binding epitopes at residues 35–42 in the amino-terminal domain (16)) were without effect (Fig. 2). To further control for nonspecific effects, one of the effective antibodies, D14, was shown to have no effect on the differentiation of the parental L6 cells (Fig. 2).

These experiments support the hypothesis that both antiparallel and parallel binding between CEA molecules are involved in the CEA-mediated myogenic differentiation block.

Structural Requirements for Myogenic Differentiation Blocking Function of CEA—Deletions and substitutions in three subdomains of the amino-terminal domain of CEA (Fig. 3) were produced by site-directed mutagenesis as described previously (16). The rationale for choosing these particular subdomains can be summarized as follows. The requirement for amino-terminal domain amino acids 32–106, deleted in mutant ΔNCEA, for the myogenic differentiation block was demonstrated previously (20). Within this deletion, subdomains 1 and 2 were implicated by the fact that mAb A20 can release the CEA-imposed myogenic differentiation block (see “Effects of Monovalent Monoclonal Anti-CEA Antibody Fragments on CEA-mediated Differentiation Blocking”) and has a binding epitope that bridges them. This epitope includes the carboxyl-terminal amino acid of subdomain 1 and the amino-terminal amino acid of subdomain 2 (16). Also, these subdomains (1 and 2) and subdomain 3 were all shown to be important in CEA-mediated intercellular adhesion in LR-73 cells; all were demonstrated to be adjacent and exposed in a three-dimensional structural model based on the known structures of CD2 (16) and CD4.3 Pooled stable transfectant clones of L6 cells expressing comparable cell surface levels, as assessed by FACS analysis (Fig. 4), were isolated for each of the deletion and substitution mutants.

Concerning subdomain 1, G30YSWYK, substitutions at the carboxyl terminus, Y34A, the more conservative Y34F, and K35A had a profound effect on the myogenic differentiation blocking activity of CEA, whereas mutation Y31A at the amino terminus had no effect (Fig. 5). As expected, deletion of the entire subdomain 1 (∆GK) also affected this function of CEA but, curiously, was not as effective as substitutions Y34A and K35A.

Similarly, the deletion of subdomain 2, N42RQII, had less effect on CEA function than some of the substitutions within this domain, notably the double mutation Q44R,I46V, for which the degree of differentiation of 100%, a reproducible effect, actually exceeded that of parental L6 cells (Fig. 6). A single amino-terminal mutation at the amino terminus of the subdomain, N42D, partially removed the differentiation blocking activity of CEA.

The mouse analogs of subdomains 1 and 2, in a crystallized soluble mouse construct of CEACAM1 consisting of the amino-terminal domain linked to one internal domain, have been shown by Tan et al. (32) to contribute to an unusual structure

3 H. U. Saragovi, unpublished data.
involving residues 35–44 that projects from the surface of the molecule, with Ile41 (Gly41 in human CEA) at its tip. This protrusion represents the binding site of murine hepatitis virus. Tyr34 in murine CEACAM1 is postulated to contribute critically to this structure. It is of interest that the above results show critical effects of Tyr34 on the differentiation blocking function of CEA, although the adjacent Lys35 residue seems to have even greater effects. It remains to be seen, of course, whether CEA has a similar structure at this site.

The third subdomain, Q80NDTG, was found to play a critical role in the CEA-mediated differentiation block, because mutation Q80A resulted in a complete loss of this function and mutation D82N, like Q44R, I46V in subdomain 2, gave 100% differentiation, thus exceeding that of the parental cells (Fig. 7). Mutations Q80R, giving 81% differentiation, and D82N, giving 100% differentiation, are of particular interest, because Q80R and D82N actually enhanced the intercellular adhesion function of CEA expressed in LR-73 cells (Fig. 8). These mutations therefore separate the intercellular adhesion and differentiation blocking functions of CEA.

Two other mutations at residue Arg84, a site shown by Sippel et al. (33) to have a marked effect on the intercellular adhesion function of CEA expressed in insect cells, had a relatively small effect on the differentiation blocking function of CEA in rat L6 myoblasts (Fig. 7).

Effects of Peptides on CEA-mediated Differentiation Block—To determine whether peptides representing the three subdomains
of CEA could release the CEA-imposed myogenic differentiation block, peptides both terminally blocked (for improved stability) and cyclized (for both improved stability and conformation) were tested by adding them to L6 (CEA) cells cultured in the presence of DM. All linear peptides were virtually ineffective (data not shown) but were effective when cyclized (Fig. 10), a conformation that is expected to mimic a β-turn configuration in the native molecule, the configuration predicted for the three subdomains. Maximum activity in releasing the differentiation block was found at a concentration of approximately 100 μM; higher concentrations of the peptides were less effective because of nonspecific toxicity. At 100 μM, cyclized peptide QNDTG released the myogenic differentiation block to the greatest extent, with an average of 36% fusion in three experiments versus 28 and 18% for cyclized peptides NRQII and GYSWYK, respectively, relative to 94% for control L6 (Neo) myoblasts. Experiments done with combinations of the three different peptides showed that there was synergy between NRQII and QNDTG; lower concentrations of these peptides (40 μM each) had the same effect as 100 μM QNDTG (data not shown).

**DISCUSSION**

Using the myogenic differentiation of rat L6 myoblasts as a model system, we investigated the structural requirements for...
the differentiation blocking activity of CEA with the goal of designing agents that were capable of releasing this activity. The basic approach was to identify small domains in which integrity was essential for function and then to test the effect of peptides representing these domains and of mAb monovalent Fab fragments that bind to them. Three such subdomains were identified, and both cyclized peptides and mAb Fab fragments were found to be effective in releasing the CEA differentiation block in L6 myoblasts.

The rationale used to focus this structure-function study was based on previous results showing that the amino-terminal and A3B3 domains of CEA were required for the differentiation blocking effect (20), thus implicating the involvement of double reciprocal bonds between antiparallel molecules on apposite cell surfaces, as observed for the intercellular adhesion function of CEA (9). A direct experiment showing that L6 (CEA) transfectants could “trans-block” the differentiation of differentiation-competent L6 (ΔNCEA) transfectants supports the notion that antiparallel CEA-CEA interactions between cells can contribute to the myogenic differentiation block. Parallel CEA-CEA interactions, probably on the same cell surface, were also implicated by the demonstration that the deletion mutant ΔNCEA, normally incapable of mediating the differentiation block, could be rendered capable of blocking L6 differentiation by cross-linking with specific antibodies. Although parallel interactions can be envisioned to improve intercellular adhesive forces by clustering antiparallel-interacting intercellular molecular pairs (the Velcro effect) (13), their relative contribution could be greater for the differentiation blocking function (34). These considerations may underlie the observation that the intercellular adhesion and differentiation blocking functions of CEA could be separated by certain amino acid substitutions in subdomain 3 that removed the differentiation blocking activity while leaving the intercellular adhesive activity intact. This result could therefore be interpreted by the suggestion that subdomain 3 is primarily necessary for parallel binding between CEA molecules. Consistent with this, peptide QNDTG, representing the third subdomain, was the most effective in releasing the CEA-imposed differentiation block.

CEA expression inhibits molecular events occurring very early in the myogenic differentiation process, notably the upregulation of the myogenic transcriptional regulator, myogenin (20). Recent evidence indicates that the molecular basis for the pan-inhibition of cellular differentiation mediated by CEA involves perturbation of the function of certain integrins (α5β1 in L6 myoblasts and human colonocytes) known to affect the earliest steps in differentiation (35–37). The results reported here showing the involvement of subdomains of the CEA mol-

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4 C. Ordoñez, A. B. Zhai, M. Fan, R. A. Screaton, C. Ilantzis, and C. P. Stanners, submitted.
ecule required for CEA-CEA binding favor a clustering model in which CEA and specific integrins inhabit the same membrane rafts defined by the glycoporphatidylinositol anchor of CEA (38). Clustering of CEA thereby causes clustering of integrin molecules, which in turn results in a change in integrin function (39). Clustering of CEA would be expected to be effected mainly by parallel interactions on the same cell surface, but antiparallel interaction may be required to initiate the process of clustering. We favor a clustering rather than dimerization model, because the effects of divalent monoclonal antibodies on integrin perturbation can be further enhanced by the addition of secondary anti-mouse antibodies. In addition, co-clustering of CEA and $\alpha_2\beta_1$ has been observed directly by confocal microscopy. This model explains why monovalent Fab fragments of specific mAbs were found to be required to release the CEA-mediated differentiation block. Whole divalent mAbs have the effect of enhancing clustering and would thus be expected to increase the tumorigenic effects of CEA.

With the knowledge that subdomains exist in the CEA molecule that can be differentially antagonized to affect the differentiation blocking function while leaving the intercellular adhesive function intact, the possibility of designing agents with functionally selective blocking activity can be entertained. Subdomain 3 was identified as such a region, but although its corresponding peptide was effective in releasing the CEA-imposed differentiation block, it was also effective in inhibiting CEA-mediated intercellular adhesion (16). Thus, although QNDTG appears to represent an experimentally useful agent with potential for medical application, further application of the methods outlined here might be expected to yield even more potent and selective agents.

Finally, even though our results to date have shown that the L6 myogenic differentiation model system used here is accurately predictive of the results obtained with more medically relevant systems, the findings bear repeating in other less experimentally convenient systems such as human Caco-2, LS-180, and SW-1222 colonocytes, in which several CEA family members are normally expressed in a regulated fashion (40). In the latter cells, deregulated overexpression of both CEA and CEACAM6 at levels observed in freshly excised human tumor colonocytes has been shown to block cell polarization and disrupt tissue architecture (22). Present findings indicate that Fab fragments of mAb A20 are capable of reversing these
tumorigenic effects, thus providing an appealing strategy for the reversal of the malignant phenotype in medically relevant situations.

Acknowledgments—We thank Dr. Lynne LeSauteur and Dr. Barbara Gour for critical advice in the design of the peptides and Dr. A. Fuks for antibodies. We thank Luisa De Marte for help in the preparation of the manuscript.

REFERENCES

1. Ruoslahti, E., and Obrink, B. (1996) Exp. Cell Res. 227, 1–11
2. Hynes, R. O., and Lander, A. D. (1992) Cell 68, 303–322
3. Edelman, G. M., and Cross, R. L. (1991) Annu. Rev. Biochem. 60, 155–190
4. Giancotti, F. G., and Ruoslahti, E. (1999) Science 285, 1028–1032
5. C. Ilantzis and C. P. Stanners, unpublished data.

5 C. Ilantzis and C. P. Stanners, unpublished data.
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J. Biol. Chem. 2003, 278:14632-14639.
doi: 10.1074/jbc.M212500200 originally published online February 5, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212500200

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