Transduction and Expression of the Human Carcinoembryonic Antigen Gene in a Murine Colon Carcinoma Cell Line

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ABSTRACT

A cell line derived from the mouse colon adenocarcinoma, MC-38, has been transduced with a retroviral construct containing complementary DNA encoding the human carcinoembryonic antigen (CEA) gene. MC-38, which forms tumors in syngeneic C57BL/6 mice, has been extensively studied as a target for active immunotherapy. Individual transduced clones that express high levels of cell surface CEA were isolated, and two clones, termed MC-38-cea1 and MC-38-cea2, were extensively characterized. The levels of CEA found on the surface of these clones were considerably higher than that found in a moderately differentiated human colon carcinoma cell line (WiDr) and were comparable to those found on the human colon carcinoma cell lines GEO and CBS (among the highest CEA-expressing cells reported). Further analysis demonstrated that the CEA expressed in the MC-38-cea1 clone had a similar molecular weight to native CEA (Mr, 180,000), but the MC-38-cea2 cell line expressed a single Mr, 70,000 glycosylated immunoreactive product. Seven anti-CEA monoclonal antibodies were found to react with both clones. The CEA gene present in the MC-38-cea2 clone was partially sequenced and was found to contain a deletion of two of the three repeated domains present in CEA. These results provide a basis for future studies to map immunodominant epitopes of CEA and to develop a syngeneic model system that may aid in the design of reagents and protocols to study active and passive immunotherapy directed against a carcinoma expressing human CEA.

INTRODUCTION

TAAs2 have been used as targets for tumor diagnosis and therapy. The ability of conjugated and unconjugated MAbs to target and kill tumors in vivo has been evaluated primarily in model systems using human tumor xenografts in athymic mice. These experiments have led to clinical trials of tumor targeting with MAbs, as well as clinical trials testing the efficacy of unconjugated and conjugated MAbs as therapeutic agents. TAAs also represent a potentially important target for active immunotherapy. For example, a system has recently been developed to evaluate the efficacy of active immunotherapy directed against the human melanoma antigen p97 (1). Initially, a cDNA clone encoding the p97 antigen was transfected into the K1735 mouse melanoma cell line, which forms pulmonary metastases when injected i.v. into syngeneic C3H/HeN mice. The p97 cDNA clone was then introduced into vaccinia virus, and infection of Chinese hamster ovary cells with this recombinant virus resulted in hepatic tumors (2). Mice were found to be protected from challenge with melanoma cells expressing this recombinant p97 following immunization with the p97 vaccinia recombinant (1). Phase I clinical trials are now being performed, performed with the recombinant p97 vaccinia recombinant.

A potential target for active immunotherapy of gastrointestinal carcinomas is CEA, which is a Mr, 180,000 glycoprotein expressed at high levels on the surface of nearly all tumors of the gastrointestinal tract (3). Model studies involving human CEA as a target of active immunotherapy cannot be carried out, however, since CEA is not expressed in rodent tumors, nor, to our knowledge, in any other animal model. In order to develop an active anti-CEA tumor therapy model system, we have transduced the MC-38 mouse colon adenocarcinoma cell line with CEA. In syngeneic C57BL/6 mice, i.v. injection of MC-38 results in pulmonary tumors (4), and i.s. injection results in hepatic tumors (5). The MC-38 tumor has previously been used in immunotherapy studies demonstrating the efficacy of lymphokine-activated killer cells in suppressing tumor cell growth (4). Tumor-infiltrating lymphocytes isolated from MC-38 tumors were effective at reducing the pulmonary micrometastases (6) as well as advanced pulmonary and hepatic metastases (7).

In this study, we present the development and analysis of two clones of transduced MC-38 cells that express high levels of human CEA on their cell surface. One of the MC-38 clones was found to contain a truncated CEA gene product that expressed a number of distinct CEA epitopes. The nature of the mutation resulting in the altered glycoprotein was examined by isolating and sequencing a portion of the CEA gene present in this clone.

MATERIALS AND METHODS

Cell Lines. The MC-38 B/6 mouse colon adenocarcinoma cell line (8) was obtained from Dr. Bernard Fox in the laboratory of Dr. Steven Rosenberg (National Cancer Institute, NIH, Bethesda, MD). This cell line was established by continuous in vitro passage of MC-38 tumor cells (5). The ecotropic retroviral packaging cell line i/-2 (9) and the amphotropic packaging cell line PA317 (10) were obtained from Dr. Robert Bassin (National Cancer Institute, NIH, Bethesda, MD). The highly differentiated human colon carcinoma cell lines GEO and CBS, as well as the moderately differentiated human colon carcinoma cell line WiDr, have been previously described (11). All cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 2 mm glutamine, 0.1 mm nonessential amino acids, 0.1 mm sodium pyruvate, and 10% newborn calf serum.

Isolation and Partial Sequencing of CEA cDNA. A cDNA library was prepared in the λ-orf8 cloning vector (12) from polyadenylate-containing mRNA isolated from the human colon tumor cell line GEO, which produces high levels of CEA. A 534-base pair PstI fragment of the CEA cDNA clone PCEA1 (13), kindly supplied by Dr. Jack Shively (City of Hope, Duarte, CA), was labeled with 32P using the random primer labeling method. Fifteen clones that hybridized to the PstI pCEA1 fragment were then screened with an oligomer complementary to a portion of the CEA leader region (5'-CAGGGGTGACCTCATGTGGGAGGAGCGGG-3') (PR1), as well as an oligomer complementary to a portion of the C-terminal hydrophobic region of CEA (5'-AACCAGCTCATCAGATGCGACAGT-3') (PR2). The oligomer PR2 hybridized to 8 of 15 clones, and PR1 hybridized with 5 clones, but none of the clones hybridized with both...
oligomers. One of the inserts (NC-1) identified by hybridization with the PR1 was subcloned and partially sequenced using the deoxynucleotide chain termination technique (14). The NC-1 insert was found to be identical to the published CEA sequence (15), starting 85 base pairs upstream of the translation start site, but only extended to the internal BamHI site present within the first repeated domain of CEA (Fig. 1a, Domain I). One of the inserts identified by hybridization with PR2 (NC-2) was subcloned, sequenced, and found to contain the internal BamHI site, the downstream coding region of CEA, along with 340 base pairs of 3' untranslated region. The absence of full-length clones may have resulted from restriction at the internal BamHI site during preparation of the library. The inserts NC-1 and NC-2 were then ligated at the internal BamHI site, and the sequence of the resultant product, extending for approximately 200 bases 5' or 3' from this site, was obtained. The sequence differed from the published sequence only at the junction of the second and third repeated domains (Fig. 1a, Domains II and III). At this position, a deletion of 3 base pairs was found, which would yield a protein lacking an alanine residue at this position. This residue has been shown to be encoded by nucleotides present within the sixth and seventh exons of the CEA gene (15); thus, this product may be the result of a splicing error.

Retroviral Expression Vector. The retroviral expression vector pBNC, provided by Dr. W. F. Anderson (National Heart, Lung, and Blood Institute, NIH, Bethesda, MD), contains the Moloney leukemia virus LTR regions. This vector was derived from the B2 vector (16) by insertion of a 1.5-kilobase EcoRI fragment of the neomycin phosphotransferase gene, isolated from the N2 vector (17), into the unique SpH1 site present in B2. The B2 vector was then digested with Sau3A and ligated to an 816-base pair fragment of the CMV promoter/enhancer (18). The CEA insert was isolated by digestion with Smal and was ligated into the unique BamHI cloning site in pBNC, which had been converted into a blunt end by the Klenow fragment of DNA polymerase.

The structure of the retroviral vector containing the CEA insert is shown graphically in Fig. 1a. Expression of the CEA gene is driven by the CMV promoter, and the neomycin phosphotransferase gene is driven by the retroviral LTR. The mature CEA glycoprotein contains an N-terminal region, followed by a set of 3 very similar repeated domains (designated I, II, and III), and a short hydrophobic C-terminal region.

Transfection and Transduction of DNA. The pB-CEA plasmid was transfected into the PA317 packaging cell line using Lipofectin (Bethesda Research Laboratories) according to the manufacturer's instructions. One wk following selection in 0.5 mg/ml of G418, the medium was changed, and the supernatant was collected for 24 h. Transduction was carried out by incubating 1 x 10^6 cells in a 6-cm dish with 1 ml of supernatant for 30 min at 37°C. Three ml of medium were then added, and 24 h later cells were removed by trypsinization and plated in 15-cm dishes. Twenty-four h later, G418 was added (0.5 mg/ml), and after selection for 2 wks in medium containing G418, cells were cloned by limiting dilution. Clones of ψ-2 cells containing the full-length CEA insert were identified by probing Southern blots of DNA isolated from those clones with the pCEA1 PstI probe. MC-38 cells could not be transduced with the recombinant ecotropic virus; therefore, PA317 cells were transduced with virus obtained from ψ-2 clones shown to contain the CEA insert. Following selection of transduced PA317 cells in 0.5 mg/ml of G418 for 3 wk, retroviral supernatants were obtained and used to transduce MC-38 cells. Following transduction, MC-38 cells were cloned by limiting dilution.

Analysis of CEA Glycoprotein Expression. Immunofluorescence assays were carried out to analyze cell surface expression of CEA as described (11). The anti-CEA monoclonal antibodies COL-1 (IgG2a), COL-4 (IgG2a), COL-6 (IgG1), COL-8 (IgG1), COL-9 (IgG2b), COL-12 (IgG1) (19), and B1.1 (IgG2a) (20), as well as the negative control murine myeloma MAb UPC-10 (IgG2a), were used as primary antibodies. Fluorescein-labeled goat anti-mouse IgG + IgM (Kirkegaard and Perry, Gaithersburg, MD) were used as the secondary antibody. Analysis was performed with a FACSscan (Becton-Dickinson Mountain View, CA) equipped with a blue laser excitation of 15 milliwatts at 488 nm. Each figure represents data obtained from analysis of 10,000 cells. Cell extracts were prepared by lysis of monolayers in nonionic detergent (24). For Western blot analysis, proteins were separated on 4 to 12% gradient sodium dodecyl sulfate-polyacrylamide gels (Novex, Encino, CA). Proteins were transferred to a PVDF membrane (0.45-μm pore size) (Millipore Corporation, Bedford, MA) using a semidry blotter (Bio-Rad, Richmond, CA) in 20% methanol, 0.039 M glycine, and 0.048 M Tris for 1 h at 1 watt/30 cm^2. Following transfer, membranes were incubated for 1 h with the MAb COL-1 (1 μg/ml). Bound antibody was detected using biotinylated goat anti-mouse antibody, followed by streptavidin-alkaline phosphatase supplied with the IMMUNOSELECT immunoblotting system, obtained from Bethesda Research Laboratories.

Radioimmunoprecipitation were carried out essentially as described (24). Briefly, 1 x 10^6 cells were seeded in a T25 flask, and the following day the medium was removed. Labeling was carried out using the RPMI-1640 Select-Amine kit, obtained from GIBCO (Grand Island, NY). The flask then received either 5 ml of medium without unlabeled leucine but with 0.5 mCi of [3H]leucine (40 to 60 Ci/mmol) (New England Nuclear, Boston, MA) or medium with 50 μg/ml of unlabeled glucose and 0.5 μCi of [3H]hlglucosamine (30 to 60 Ci/mmol) (New England Nuclear) for 18 h. Cell extracts were then prepared, and immunoprecipitations were carried out with 1 ml of tissue culture supernatant containing either the anti-CEA MAB COL-1 or the negative control MAB RPC-5 (IgG2a). The immunoprecipitates were analyzed on a 10% sodium dodecyl sulfate-polyacrylamide gel, which was stained with 0.25% Coomassie blue, destained, and incubated with ENHANCE (New England Nuclear) according to the manufacturer's instructions. The gels were then dried and subjected to autoradiography.

Amplification of DNA by PCR. Two primers were used to amplify the human CEA gene from the genomic DNA of transduced MC-38 clones. The first (5'-AACCTCAAGCTCTTCTCCACA) was located in the 5'-untranslated region of the CEA gene, and the second (5'-AAGCAATTTTTAGCTGTAG) was complementary to a portion of the 5'-untranslated region of the CEA gene. The PCR was carried out using 1 μg of genomic DNA and 500 ng of each of the two primers in PCR buffer (10 mM Tris(pH 8.3)-50 mM KCl-1.5 mM MgCl2) with 5 units of Taq DNA polymerase (Bethesda Research Laboratories). Primers were annealed for 2 min at 55°C, extension was carried out for 3 min at 70°C, and denaturation was carried out for 2 min at 94°C. Amplification was carried out for 30 cycles.

RESULTS

CEA Glycoprotein Expression in Transduced MC-38 Cells. Following selection in G418, transduced MC-38 cells were...
cloned by limiting dilution, and the expression of CEA on the surface of transduced clones was then analyzed in an immunofluorescence assay. Nine clones that expressed high levels of CEA were identified, and two (MC-38-cea1, MC-38-cea2) were chosen for more extensive analysis. The levels of cell surface expression of CEA found on clones MC-38-cea1 and MC-38-cea2 (Fig. 2, a and b) were comparable to those found on the GEO and CBS human colon carcinoma cell lines (Fig. 2, d and e). GEO and CBS are two human colon tumor cell lines that express levels of CEA which are substantially higher than those found in most human colon carcinoma cell lines (11). Cell surface expression of CEA on the MC-38-cea1 and MC-38-cea2 cell lines was considerably higher than that found on the surface of WiDr, a human colon carcinoma cell line expressing moderate levels of CEA (Fig. 2f).

Quantitation of CEA expression in cell extracts was then carried out using the Roche enzyme immunoassay, which is a double-determinant assay utilizing two anti-CEA MAbs. Levels of CEA in MC-38-cea1 and MC-38-cea2 cell extracts were higher than that found in an extract prepared from the WiDr cell line (Table 1). Much higher levels were found in protein extracts prepared from the GEO and CBS cell lines (Table 1). CEA levels were then quantitated using the Abbott radioimmunoassay, which is a double-determinant assay containing two additional anti-CEA MAbs. When the MC-38-cea1 extract was assayed with the Abbott assay, values similar to that obtained with the Roche assay were obtained, with the exception of the MC-38-cea2 extract, which was negative in the Abbott assay. This indicates that at least one of the epitopes present on the native CEA molecule is not expressed on the transduced gene product expressed in MC-38-cea2 (to be discussed below).

Expression of CEA Epitopes. The expression of additional CEA epitopes on the MC-38-cea1 and MC-38-cea2 cell lines was then examined by carrying out a cell surface immunofluorescence assay using a number of MAbs (Fig. 3). The epitopes defined by the MAbs COL-1, 6, 8, and 9 are found on CEA but not on NCA, whereas those defined by the MAbs COL-4, 12, and B1.1 are found on both CEA and NCA (25). All of the anti-CEA MAbs reacted with the two transduced clones; however, the level of expression of these epitopes appeared to be higher on the MC-38-cea1 clone than on the MC-38-cea2 clone. The ratio of the mean fluorescence intensity found on MC-38-cea1 versus that found on MC-38-cea2 varied from 1.3 to 2.5 with COL-9 to 2.5 with COL-8 (Fig. 3), further indicating that MC-38-cea2 may express an altered CEA gene product. The MAb B6.2, which reacts with NCA but not CEA (21), failed to react with either of the transduced clones.

Characterization of Transduced CEA Gene Products. The size of the CEA gene products expressed by the transduced cells was then examined in a Western blot assay. The MC-38-cea1 cell line expressed the expected M, 180,000 glycoprotein (Fig. 4, Lane 2), similar to that found in the WiDr cell line (Lane 1). The immunoreactive product present in the MC-38-cea2 cell line, however, had a molecular weight of approximately 70,000 (Lane 3). Examination of seven additional clones indicated that the size of the CEA gene products frequently varied in clones of transduced cells. Four clones expressed a single M, 70,000 immunoreactive product, two expressed a single M, 140,000 product, and one expressed two products with molecular weights of 180,000 and 140,000 (data not shown).

The molecular weight of the MC-38-cea2 product was very similar to that of the predicted polypeptide backbone of CEA (M, 72,000) and could, therefore, have resulted from the lack of processing or from the post-translational modification of the polypeptide backbone. The size of the CEA gene products frequently varied in clones of transduced cells. Four clones expressed a single M, 70,000 immunoreactive product, two expressed a single M, 140,000 product, and one expressed two products with molecular weights of 180,000 and 140,000 (data not shown).

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Table 1 CEA expression in cell extracts

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Description</th>
<th>Assay 1*</th>
<th>Assay 2*</th>
</tr>
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<tbody>
<tr>
<td>GEO</td>
<td>Human colon adenocarcinoma</td>
<td>8400</td>
<td>4400</td>
</tr>
<tr>
<td>CBS</td>
<td>Human colon adenocarcinoma</td>
<td>1300</td>
<td>3200</td>
</tr>
<tr>
<td>WiDr</td>
<td>Human colon adenocarcinoma</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>A 375</td>
<td>Human melanoma</td>
<td>Negative*</td>
<td>Negative*</td>
</tr>
<tr>
<td>MC-38-cea1</td>
<td>Mouse colon adenocarcinoma</td>
<td>230</td>
<td>150</td>
</tr>
<tr>
<td>MC-38-cea2</td>
<td>Mouse colon adenocarcinoma (M, 70,000 CEA)</td>
<td>57</td>
<td>Negative</td>
</tr>
<tr>
<td>MC-38</td>
<td>Mouse colon adenocarcinoma</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
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* Quantitation of CEA was carried out using the Roche enzyme immunoassay kit (Assay 1) and the Abbott radioimmunoassay kit (Assay 2).

* Less than 2.5 ng/mg.

![Fig. 2. Cell surface expression of CEA in transduced cell lines. The expression of CEA was examined by carrying out immunofluorescence assays with the anti-CEA MAb COL-1 (—) or the control antibody UPC-10 (---). The mean fluorescence intensities obtained with the control MAb UPC-10 and the anti-CEA MAB COL-1, respectively, are given in parentheses following the cell designations. The fluorescence intensity was plotted on a linear scale. The cells which were analyzed are as follows: A, MC-38-cea1 (30.5, 95.2); B, MC-38-cea2 (21.1, 100); C, MC-38 (32.2, 29.2); D, GEO (43.4, 102); E, CBS (60.8, 109); and F, WiDr (51.2, 70.7).](image)

![Fig. 3. Expression of CEA epitopes in transduced cell lines. A cell surface immunofluorescence assay was carried out on the MC-38-cea1 cell line (---) and the MC-38-cea2 cell line (----) with the MAbs COL-1 (4) (180, 106), COL-4 (B) (320, 201), COL-6 (C) (250, 152), COL-8 (D) (164, 65), COL-9 (E) (165, 123), COL-12 (F) (151, 69), B1.1 (G) (253, 124), B6.2 (H) (29, 43), and UPC-10 (I) (26, 38). The numbers in parentheses represent the mean fluorescence intensity obtained when using these MAbs to stain the MC-38-cea1 and MC-38-cea2 clones, respectively. The fluorescence intensity was plotted on a logarithmic scale.](image)

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Analysis of Integrated CEA Gene. In order to characterize the mutation present in MC-38-cea2, a Southern blot was carried out with DNA isolated from this clone, as well as from the MC-38-cea1 clone. Digestion with BamHI should release two fragments that hybridize to the CEA probe: a 1-kilobase fragment containing the 5' end of CEA as well as a unique fragment whose size is dependent on the integration site of the vector (Fig. 1a). Unique CEA-hybridizing fragments of 4.5 and 6.0 kilobases were seen following BamHI digestion of DNA from MC-38-cea1 and MC-38-cea2, respectively (Fig. 6, Lanes 2 and 3). The 1-kilobase fragment, however, was present in the BamHI digest of MC-38-cea1 but not MC-38-cea2 DNA, indicating that a portion of the CEA gene had been deleted in MC-38-cea2. Additional analysis has indicated that the high-molecular-weight band seen in the BamHI digest of MC-38-cea1 DNA is present in digests of MC-38 and MC-38-cea2 DNA (data not shown) and may therefore result from hybridization of this probe with a homologous mouse gene (27). Thus, only one copy of the human CEA gene appears to be present in each of the two cloned cell lines. In order to determine the size of this deletion, DNA isolated from the MC-38-cea2 clone was digested with EcoRI, which should release the entire CEA insert on a single 4.4-kilobase fragment. A 4.4-kilobase band that hybridized to the CEA probe was found following digestion of MC-38-cea1 DNA with EcoRI (Lane 5), whereas a 3.3-kilobase band was seen with the EcoRI digest of MC-38-cea2 DNA (Lane 6). The high-molecular-weight bands seen in the EcoRI digest appear to be shared with the parental MC-38 cell line and, thus, again may result from hybridization of the CEA probe to homologous mouse genes. To further analyze the deletion that had taken place, digestion was carried out with EcoRI plus BamHI, which should release the CEA insert on two fragments of 1.5 and 0.9 kilobases. Digestion of DNA from...
the MC-38-cea1 cell line with these enzymes gave rise to two bands of the expected size (Lane 8), whereas digestion of MC-38-cea2 DNA yielded a single 1.4-kilobase band that hybridized with the CEA probe (Lane 9). These results indicated that a deletion of approximately 1.0 kilobase had occurred within the CEA gene present in the MC-38-cea2 cell line.

The CEA gene present in MC-38-cea2 was then isolated by using the polymerase chain reaction to amplify genomic DNA isolated from this clone. A 1.1-kilobase fragment was obtained from the MC-38-cea2 cell line using oligonucleotide primers that flank the CEA coding region, and this product was subcloned and partially sequenced. The sequence of approximately 150 nucleotides at the 5' and 3' ends of the amplified product corresponded to the published sequence (15), indicating that no deletions had taken place at either end of the integrated CEA gene. The sequence of the MC-38-cea2 clone in the region following the A'-terminal domain corresponded to that of the MC-38-cea1 cell line with these enzymes gave rise to two bands of the expected size (Lane 8), whereas digestion of MC-38-cea2 DNA yielded a single 1.4-kilobase band that hybridized with the CEA probe (Lane 9). These results indicated that a deletion of approximately 1.0 kilobase had occurred within the CEA gene present in the MC-38-cea2 cell line.

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DISCUSSION

Studies have demonstrated expression of the CEA gene in L-cells, as well as Chinese hamster ovary cells (28), but the studies reported here represent the first example of expression of the human CEA gene product in a mouse tumor cell line, and in particular a colonic epithelial tumor cell. Following transduction of a mouse colon adenocarcinoma cell line (MC-38) with the CEA gene, clones were obtained that express levels of cell surface CEA comparable to that found in GEO and CBS cells, two human colon tumor cell lines that express high levels of CEA. However, the levels of CEA found in total cell extracts of the transduced clones were substantially lower than the levels found in the GEO and CBS tumor cell lines. The failure of GEO and CBS to express significantly higher levels on their cell surface may reflect increased turnover of CEA in these cells. Alternatively, since CEA is linked to the cell membrane through a glycosyl phosphatidylinositol group (26), the availability of sites on the surface of these cells for linkage to CEA may limit the cell surface expression of CEA in GEO and CBS.

The transduced product observed in the MC-38-cea2 cell line is a glycosylated protein with a molecular weight of approximately 70,000, whereas the normal CEA gene product has a molecular weight of 180,000. Southern blot analysis indicated that a deletion of the CEA sequence had occurred, and sequencing of the CEA gene isolated from the MC-38-cea2 by the polymerase chain reaction indicated that this product contained only one copy of the domain present in three copies in the normal CEA gene product. In spite of the loss of two domains, the MC-38-cea2 product expressed the epitopes recognized by all of the anti-CEA MAbs tested, with the exception of at least one of the MAbs in the Abbott CEA radioimmunoassay kit. Antibodies that react with the MC-38-cea2 product include four MAbs which recognize epitopes present on CEA but not on NCA, as well as three MAbs that react with epitopes shared between CEA and NCA (25). Some of these epitopes may be represented more than once on the normal CEA gene product, since approximately 70% of the amino acid sequence is conserved among the three repeated domains. The loss of one or two of the repeated domains would not be expected to affect recognition by these antibodies. Some of these epitopes may also be present within the N-terminal domain of CEA, which appears to be unaltered in the MC-38-cea2 product.

In terms of appropriateness for an in vivo model for a CEA-expressing tumor, there are three points to consider: total cellular CEA content, expression of CEA on the cell surface, and amount of CEA shed. For comparison, we have used human colon cancer cell lines expressing high (GEO) and moderate (WiDr) levels of CEA. As shown in this study, the MC-38-cea1 and MC-38-cea2 cells express as much cell surface CEA as GEO, and they express significantly more cell surface CEA than the WiDr cell line. Additional experiments have shown that transduced MC-38 cells shed relatively low amounts of CEA, comparable to the amount shed by the WiDr cell line, but considerably less than that shed by the GEO cell line (data not shown). One could speculate that the turnover of CEA in MC-38 cells may be comparable to that seen in moderately expressing cell lines such as WiDr. Therefore, in comparison to most human carcinomas, we believe the transduced MC-38 cells express substantial amounts of cell surface CEA and shed low levels of CEA, which should make this an appropriate target for immunotherapy.

The generation of a mouse tumor cell line which expresses human CEA gene should now allow development of an in vivo model system to test anti-CEA therapies. Using these cells, active immunotherapy, as well as antibody-mediated therapy directed against CEA, can be evaluated in an animal model of disseminated disease, since MC-38 cells form pulmonary tumors following i.v. injection of tumor cells expressing CEA-TRANSDUCED CARCINOMA CELLS. Antigen recognition by these antibodies. Some of these epitopes may be served among the three repeated domains. The loss of one or two of the repeated domains would not be expected to affect recognition by these antibodies. Some of these epitopes may also be present within the N-terminal domain of CEA, which appears to be unaltered in the MC-38-cea2 product.

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ACKNOWLEDGMENTS

We would like to thank Dianne Poole for her excellent technical assistance.

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