Human Autoantibodies to Carcinoembryonic Antigen (CEA) Induced by a Vaccinia-CEA Vaccine

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ABSTRACT

Carcinoembryonic antigen (CEA) is a well-characterized oncofetal glycoprotein whose overexpression by human adenocarcinomas has been a target for cancer immunotherapy. Limited information is available regarding the ability of patients to mount an antibody response to this self-antigen following vaccination. Recombinant vaccinia viruses encoding full-length or internally deleted cDNAs for human CEA were used to vaccinate 32 patients with CEA-expressing adenocarcinomas, predominantly of colorectal origin. CEA-specific autoantibodies were induced by vaccination in 7 of 32 patients. None of the patients had CEA antibodies detected before vaccination. CEA specificity of the antibodies initially identified by ELISA was confirmed by competitive inhibition analysis as well as recognition of recombinant CEA produced in baculovirus-infected insect cell cultures and human cell cultures by Western blot. The CEA autoantibodies were predominantly IgG1, with a minority of patients also demonstrating IgM autoantibodies. CEA antibodies were of low titer and low avidity, based on competitive inhibition assays. These autoantibodies did not affect clinical serum CEA protein quantitation. Furthermore, elevated serum CEA levels commonly encountered in patients with advanced adenocarcinoma did not hinder detection of low avidity polyclonal CEA antibodies. CEA antibodies such as those induced in these pilot trials are projected to have modest antitumor activity. Thus, additional Phase I/II trials of recombinant vaccinia-CEA with alternative prime-boost approaches and/or augmentation strategies are warranted in an effort to enhance the frequency and avidity of CEA-specific autoantibodies and cytolytic T cells before Phase III trials.

INTRODUCTION

CEA is a Mr 180,000 membrane-anchored glycoprotein expressed on the great majority of colorectal, gastric, and pancreatic carcinomas as well as approximately 50% of breast cancers and 70% of non-small cell lung cancers (1). CEA is also expressed, to a limited extent, in normal colonic mucosa and fetal digestive organs. The immunogenicity of CEA in normal human tissue or in CEA-expressing adenocarcinomas remains controversial. Several studies from the late 1970s and early 1980s suggest the presence of circulating free anti-CEA antibodies or CEA-immune complexes in adenocarcinoma patients (2–5). However, other reports suggest that such observations may be artifactual (6–8).

Recombinant vaccinia viruses encoding full-length or internally deleted cDNAs for human CEA are replication competent and direct cell surface expression of CEA (9). Immunization with rV-CEA has induced CEA-specific humoral and cellular immune responses in mice and nonhuman primates as well as protection of mice against challenge with syngeneic colon carcinoma cells expressing human CEA (10, 11). However, it is important to emphasize that human CEA is a foreign antigen in both mice and nonhuman primates, whereas humans appear to be immunologically tolerant to CEA related to its expression in fetal and normal adult tissues. To provide a more suitable model for studying various immunotherapy strategies directed against this tumor self-antigen, transgenic mice that express human CEA with a tissue distribution similar to that of humans have been developed (12). Immunization of these CEA transgenic mice with rV-CEA induced modest titer of IgG and IgM CEA antibodies, CEA-specific helper and cytotoxic T-cell responses, and protection against challenge with CEA-expressing tumor cells (13). Anti-CEA IgG titers generated by repetitive rV-CEA immunization in CEA transgenic mice were approximately 40-fold lower than those measured in corresponding CEA-negative littermates, a difference that may be related to tolerance in the former group.

The first clinical trial of rV-CEA was conducted by the Navy Oncology Branch of the NCI in 26 patients with metastatic adenocarcinoma (14, 15). No primary lymphoproliferative responses to soluble CEA protein were observed, and no antibody responses to CEA were reported (14, 15). However, human leukocyte antigen-A2-restricted cytolytic T-cell lines re-
responsive to specific CEA peptides could be derived by prolonged in vitro culture of peripheral blood lymphocytes from patients after vaccination (15).

Subsequently, our group completed a NCI-sponsored Phase I clinical trial using rV-CEA in 20 patients with widely metastatic and predominantly colorectal adenocarcinoma (16). Vaccination serum CEA levels ranged from 1–3358 ng/ml among these patients. This study will hereafter be referred to as UAB 9619. In this trial, the recombinant vaccinia virus encoded the full-length cDNA for CEA (M, 180,000). Patients were randomly assigned to receive the vaccine by either standard intradermal injection or s.c. jet injection. All patients received two doses of either 10^7 or 10^8 pfu of rV-CEA at a 4-week interval. Toxicity was limited to modest local inflammation at the inoculation site and low-grade fever and fatigue affecting a minority of patients. No evidence of CEA-specific lymphoproliferation, interleukin-2 release, or delayed type hypersensitivity was observed (16).

Our group recently completed a second NCI-sponsored Phase I clinical trial using rV-CEA in 12 patients with Dukes' stage C or D colorectal adenocarcinoma rendered free of detectable disease by standard treatment methods. This study will hereafter be referred to as UAB 9501. In this trial, the recombinant vaccinia virus encoded a M, 70,000 truncated cDNA containing an in-frame deletion of two of the three CEA repeated domains (17). One group of six patients received 4 × 10^7 pfu of rV-CEA by scarification at weeks 0 and 8. A second group of six patients received the same dose and schedule of rV-CEA plus 300 mg/m² cyclophosphamide i.v. 3 days before each vaccine dose. No toxicities were observed except for modest local inflammation at the inoculation site and mild nausea attributable to cyclophosphamide. Whereas T-cell responses to vaccinia virus were observed, no lymphoproliferative responses to soluble CEA occurred before or after rV-CEA vaccination.

In this report, we present the analysis of serological immune responses to CEA among the 32 patients in the two trials of rV-CEA performed by our group. The data provide the first evidence for induction of anti-CEA autoantibodies in patients after rV-CEA vaccination.

**MATERIALS AND METHODS**

**Reagents.** Baculovirus recombinant human CEA (M, 120,000) was kindly provided by MicroGeneSys (Meriden, CT) and is referred to as “baculovirus rCEA.” Human rCEA (M, 180,000) produced in a stably transfectant human cell line was purchased from Vitro Diagnostics (Littleton, CO) and is referred to as “human rCEA.” Mouse monoclonal anti-CEA antibody COL-1 was kindly provided by Dr. Jeffrey Schlom (NCI, Bethesda, MD; Ref. 18).

**ELISA for Anti-CEA Antibodies.** To detect anti-CEA antibodies, microtiter plates were coated overnight with 100 ng/well of baculovirus rCEA protein (MicroGeneSys). The plates were blocked for 90 min at 37°C with 1% pig skin gelatin and 3% nonfat dry milk in PBS (referred to as milk buffer with gelatin). The plates were incubated with patient or normal donor sera diluted in milk buffer without gelatin overnight at 4°C. Plates were washed, and antibody binding was detected with HRP-conjugated goat antihuman IgG (heavy and light chain) antiserum (1:5000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). This antiserum binds human IgA, IgG, and IgM by virtue of light chain recognition. HRP activity was detected by incubation with substrate, and absorbance was read at 405 nm. A positive anti-CEA antibody response was defined as a posttreatment absorbance greater than twice the pretreatment absorbance for the individual patient and greater than the mean plus two SDs of 10 normal donor sera assayed at the same dilution.

**Competitive ELISA with Patient Antisera.** For competitive inhibition assays, patient sera obtained 5–8 weeks after primary immunization were selected on the basis of producing the highest absorbance in the ELISA for direct CEA binding. These sera were diluted 1:30 in milk buffer with or without inhibitor protein, added immediately to microtiter plates coated with baculovirus rCEA, and incubated overnight at 4°C to allow antigen-antibody interactions to reach equilibrium. The remainder of the ELISA was conducted as described above. Inhibitor proteins consisted of baculovirus rCEA at 100 ng/ml or 100 μg/ml, human rCEA at 100 ng/ml or 100 μg/ml, BSA at 100 μg/ml as a negative control for nonspecific protein inhibition.

The inhibitor concentration of 100 μg/ml was selected to provide a 100-fold excess of soluble CEA compared to the quantity of CEA used to coat each well. Inhibition with 100 μg/ml baculovirus rCEA uniformly reduced the ELISA absorbance in wells coated with the same CEA protein by >75% and was used to define the background absorbance due to nonspecific binding. The percentage of inhibition was calculated by the following formula:

\[
\frac{A - B}{A - C} \times 100\%
\]

where A is the absorbance produced by serum alone, B is the absorbance produced by serum plus an inhibitor (BSA or human rCEA), and C is the background absorbance due to nonspecific binding.

**Western Immunoblot.** Either 10 μg of baculovirus rCEA (MicroGeneSys) or 30 μg of human rCEA (Vitro Diagnostics) were preboiled in 2× SDS sample buffer and loaded onto an 8% SDS-polyacrylamide gel. After electrophoresis and electrophoresis onto a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA), the membrane was blocked for 1 h at room temperature with 5% nonfat dry milk in PBS. The membrane was then mounted on the Mini-Protein II Multiscreen apparatus (Bio-Rad Laboratories), and each lane was filled with 200 μl of patient sera diluted 1:3 or 1:10. Positive controls consisted of the COL-1 anti-CEA monoclonal antibody (18) or monkey polyclonal anti-CEA serum (19) diluted 1:100. All dilutions were in 5% nonfat dry milk in PBS. After incubation overnight at 4°C on a rotator, the membrane was washed by immersing the whole apparatus in 3 liters of PBS at room temperature for 20 min with shaking. The wash process was repeated once and followed by one wash with Tris-buffered saline [150 msi NaCl, 50 msi Tris-Cl (pH 7.5)]. Membrane-bound antibodies were detected by HRP-conjugated goat antihuman IgG antiserum for COL-1 lanes or by HRP-conjugated goat antihuman IgG antiserum (Jackson ImmunoResearch Lab-
oratories, Inc.) for lanes receiving monkey or patient sera. Each lane was incubated for 1 h at room temperature with the appropriate secondary antibody diluted 1:4000 in 5% nonfat dry milk in PBS. The membrane was washed four times in 200 ml of Tris-buffered saline for 10 min each at room temperature on a rotator. The Opti-4CN substrate kit (Bio-Rad Laboratories), was used for color development, whereas Chemiluminescence Reagent Plus (NEN Life Science Products, Inc., Boston, MA) was used for luminescence detection and exposure to X-ray film.

**Antibody Isotyping Assay.** The immunoglobulin classes comprising anti-CEA antibody responses were examined by the ELISA method described above with one alteration. CEA-specific antibodies captured by plate-bound antigen were detected with a panel of three secondary antisera (Jackson ImmunoResearch Laboratories, Inc.), each at 1:5000 dilution: (a) goat antihuman IgG (Fc) (IgG specific); (b) goat antihuman IgM; or (c) goat antihuman IgA. Relative quantities were depicted as absorbance values of sera obtained before and after immunization using each detection antisera. A positive result was defined as postimmunization absorbance at least 2-fold greater than preimmunization absorbance. IgG subclasses were assessed using HRP-conjugated anti-IgG1, anti-IgG2, anti-IgG3, and anti-IgG4 antibodies (The Binding Site, San Diego, CA). Standard curves were generated with human myeloma proteins IgG1, IgG2, IgG3, and IgG4, all with κ light chains (The Binding Site), and patient serum results were normalized against these subclass standards. A positive IgG subclass response was defined as posttreatment binding greater than the mean plus two SDs of the pretreatment binding for the seven patients tested.

**Competitive ELISA with Monkey Anti-CEA Sera and Monoclonal Anti-CEA.** Microwell plates were coated overnight with 200 ng/well human rCEA (Vitro Diagnostics) and blocked with milk buffer with gelatin. Polyclonal monkey anti-CEA sera were generated by immunizing pig-tailed macaques with plasmid DNA encoding human CEA by i.m. injection or particle bombardment of the skin as described previously (19). Antisera following primary immunization or first boost were selected to provide relatively low titer, low affinity anti-CEA antibodies resembling those observed in the vaccinated patients. Postimmunization sera from three macaques and COL-1 anti-CEA monoclonal antibody at 600 μg/ml in neat normal mouse serum were diluted 1:100 in milk buffer without gelatin, followed by the addition of human rCEA to concentrations ranging from 1–100,000 ng/ml (corresponding to 10^2–10^7 ng CEA/ml neat serum after correcting for the 1:100 dilution). The diluted sera containing soluble human rCEA were immediately added to ELISA wells and incubated overnight at 4°C to allow antigen-antibody interactions to reach equilibrium. The remainder of the ELISA was conducted as described above with peroxidase-conjugated goat antihuman IgG (heavy and light chain) and antimouse IgG (heavy and light chain), each at 1:5000 (Jackson ImmunoResearch Laboratories), to detect monkey antisera and COL-1, respectively.

**Serum CEA Protein Quantitation.** Selected patient sera obtained 5–8 weeks after primary immunization were spiked with human rCEA for final concentrations ranging from 0–100 ng/ml; normal donor sera were similarly spiked as controls. The sera were assayed for CEA content in the hospital clinical laboratory using the AXSYM system (Abbott Laboratories, Abbott Park, IL). This system uses mouse monoclonal anti-CEA-coated microparticles to capture soluble CEA and uses an alkaline phosphatase-conjugated mouse monoclonal anti-CEA antibody for detection.

**RESULTS**

**Anti-CEA Antibody Response by ELISA.** Sera from 20 metastatic adenocarcinoma patients immunized with rV-CEA on protocol UAB 9619 were analyzed for anti-CEA antibodies by ELISA. None of these patients demonstrated evidence of anti-CEA antibodies before immunization. Four patients developed anti-CEA antibodies after immunization with rV-CEA, as shown in Fig. 1A. Patients 1, 3, and 16 received rV-CEA by s.c. jet injection, whereas patient 11 received the vaccine by standard intradermal injection. Anti-CEA antibodies developed in one patient after primary immunization and developed in four patients within 1 week after a boost.

Sera from 12 colorectal carcinoma patients immunized with rV-CEA in the adjuvant setting on protocol UAB 9501 were also analyzed for anti-CEA antibodies by ELISA. Again, none of these patients demonstrated anti-CEA antibodies before immunization, but three patients developed anti-CEA antibodies...
Table 1  Inhibition of patient antiserum binding to baculovirus rCEA: percentage inhibition for patient samples

Values shown are the percentage inhibition calculated as described in “Materials and Methods.”

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>UAB 9619</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pt 1 Pt 3 Pt 11 Pt 16</td>
</tr>
<tr>
<td>BSA (100 μg/ml)</td>
<td>2 3 14</td>
</tr>
<tr>
<td>Human rCEA (100 μg/ml)</td>
<td>80 69 82 78</td>
</tr>
<tr>
<td>Human rCEA (100 ng/ml)</td>
<td>0 4 1 0</td>
</tr>
<tr>
<td>Baculovirus rCEA (100 ng/ml)</td>
<td>0 12 6 11</td>
</tr>
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</table>

*Pt, patient.

after immunization with rV-CEA. As shown in Fig. 1B, anti-CEA antibody responses peaked 8 weeks after primary immunization and remained detectable 8 weeks after a boost. Patients 2 and 4 received the vaccine alone, and patient 10 was pretreated with cyclophosphamide. Failure of the boost to increase the anti-CEA antibody titers may be due to host immune response to the vaccinia virus vector (16).

**Competitive ELISA with Patient Antisera.** We next sought to confirm that binding of patient antiserum to ELISA wells coated with baculovirus rCEA represented true CEA reactivity and not reactions with trace viral or insect cell contaminants within the CEA protein preparation. For this purpose, we examined the ability of human rCEA produced in a stably transfected human cell line to competitively inhibit reactivity with baculovirus rCEA (Table 1). Human rCEA and an irrelevant control protein, BSA, were each added at 100 μg/ml, corresponding to a 100-fold excess compared to the amount of CEA used to coat each ELISA well. Excess soluble BSA as a control for nonspecific inhibition reduced serum antibody binding to CEA by only 2–15%. However, soluble human rCEA inhibited binding by 69–82%. These results indicate that patient antisera recognized epitopes shared between human rCEA and baculovirus rCEA, and not contaminants in the CEA preparation. This result was expected because the patients were immunized with rV-CEA and thus had no known exposure to contaminants that might be present in the recombinant protein preparations.

We also sought to examine the ability of soluble CEA protein at concentrations that can be encountered in the sera of patients with metastatic adenocarcinoma to inhibit detection of anti-CEA antibodies by ELISA. For this purpose, rCEA inhibitors were added at 100 ng/ml, corresponding to 3000 ng/ml CEA in undiluted serum. Both human rCEA and baculovirus rCEA produced mean percentage inhibitions of less than 10% (Table 1), indicating that the ELISA used for the detection of serum CEA autoantibodies would not be compromised by the patient’s circulating CEA levels.

**Western Immunoblot.** Sera from the four metastatic adenocarcinoma patients (protocol UAB 9619) demonstrating CEA autoantibody responses by ELISA reacted with baculovirus rCEA protein by Western blot assay. As shown in Fig. 2, the COL-1 anti-CEA monoclonal antibody detected a diffuse band at approximately M₄ 120,000, corresponding to the molecular weight of partially glycosylated baculovirus rCEA (20). Postimmunization sera from all four patients produced a similar band at approximately M₄ 120,000, whereas all preimmunization sera were negative. Fig. 2 provides representative data from two of the four patients.

Western blot analysis was also performed with human rCEA using sera from all seven patients demonstrating anti-CEA antibody responses by ELISA. As shown in Fig. 3, polyclonal monkey anti-CEA serum (19) produced a clear band at approximately M₄ 180,000, corresponding to the molecular weight of fully glycosylated human CEA (1). A panel of eight normal human sera and preimmunization sera from all seven patients were negative, producing no detectable bands. Fig. 3 provides data from three representative patients whose postimmunization sera demonstrated a band at approximately M₄ 180,000.

**Isotopy.** The immunoglobulin class analysis of anti-CEA antibodies from the highest titer sera of individual patients induced by immunization with rV-CEA is illustrated in Fig. 4. IgG was the dominant class of anti-CEA antibody occurring in all seven patients. IgM responses were observed in three of seven patients, and a modest IgA response was seen in only one of seven patients. IgG anti-CEA antibodies were almost exclusively of the IgG1 subclass, with levels as high as 3 μg bound per milliliter serum (Table 2). Modest levels of IgG3 anti-CEA antibodies occurred in two of seven patients, whereas no IgG2 or IgG4 anti-CEA antibodies were detected.
Competitive ELISA with Polyclonal Anti-CEA Sera and Monoclonal Anti-CEA.

We next sought to examine the potential for circulating CEA to diminish detection of circulating CEA autoantibodies by acting as a competitive inhibitor in the ELISA assay. As a model, polyclonal monkey anti-CEA sera were selected as described in “Materials and Methods” to provide relatively low titer, low affinity, anti-CEA antibodies to resemble those observed in our patients. This analysis used monkey rather than patient sera because elevated human CEA levels in patient sera would have confounded the experiment. Fig. 5 illustrates the binding of 1:100 dilutions of these antisera to solid-phase human rCEA in the presence of varying concentrations of soluble human rCEA. For the three monkey antisera, the mean ± SE IC_{50} is 62,000 ± 10,000 ng CEA/ml neat serum. Alternatively, a serum CEA concentration of 10^5 ng/ml is required to decrease the ELISA absorbance comparable to a half-log decrease in monkey antibody concentration (data not shown). For comparison, Fig. 5 also provides a competitive inhibition curve for COL-1 anti-CEA monoclonal antibody with varying concentrations of human rCEA. The IC_{50} decreased to 1,200 ng CEA/ml neat serum when this monoclonal antibody with high affinity for soluble CEA was used.

Serum CEA Quantitation.

We next examined the influence of circulating anti-CEA antibodies on the quantitation of serum CEA protein. Human rCEA protein was added at 0, 10, or 100 ng/ml to a panel of normal donor human sera, and the mean ± SD for the serum CEA was 0.6 ± 0.1, 11.8 ± 0.9, and 109 ± 10 ng/ml, respectively. When CEA was added over the same range of concentrations to sera from three patients with anti-CEA antibodies after rV-CEA immunization in the adjuvant setting, the mean ± SD for the serum CEA determinations were similar (0.5 ± 0.4, 12.2 ± 2, and 119 ± 11 ng/ml, respectively). Finally, the sera of three patients with anti-CEA antibodies after immunization with rV-CEA for widespread metastases were examined. These patients had baseline serum CEA levels ranging from 27–47 ng/ml. The addition of 100 ng/ml human rCEA to these sera produced a mean ± SD increment of 115 ± 6 ng/ml. Therefore, it can be concluded that...
the relatively low titer, low affinity, anti-CEA antibodies induced in these patients by rV-CEA immunization have no demonstrable influence on the clinical quantitation of serum CEA levels.

**DISCUSSION**

Limited information is available regarding the ability of patients to mount an antibody response to CEA after vaccination. CEA antibody responses have been reported among colorectal carcinoma patients vaccinated with baculovirus rCEA protein with or without granulocyte macrophage colony-stimulating factor (21). However, these data are difficult to interpret because anti-CEA antibodies were measured by ELISA using plates coated with the same baculovirus rCEA protein used to vaccinate the patients. Thus, vaccination could have induced antibody responses to baculovirus and/or insect cell contaminants that would compromise the specificity of ELISA results. The only other report of CEA antibody induction in patients after vaccination involves an anti-idiotype vaccine mimicking a specific epitope of CEA (22–25). Thirteen of 24 patients with advanced colorectal carcinoma demonstrated CEA-specific antibody responses after repeated administration of the aluminum hydroxide-precipitated anti-idiotype vaccine (23). Recently, 32 of 32 colon carcinoma patients receiving the anti-idiotype vaccine in the postsurgical adjuvant setting developed CEA antibody responses that generally peaked after the sixth dose (25). The patients in the present study received only two doses of rV-CEA, which may partially explain the low frequency, titer, and avidity of anti-CEA antibody responses.

Here, we provide the first demonstration that CEA autoantibodies can be induced by vaccination with a recombinant vaccinia virus. CEA antibodies were induced after vaccination with rV-CEA in 4 of 20 patients with widely metastatic adenocarcinoma and in 3 of 12 patients with a history of colorectal carcinoma in the adjuvant setting. None of the patients had detectable preexisting CEA antibodies. CEA specificity of the antisera was confirmed by competitive inhibition analysis as well as by recognition of baculovirus rCEA and human rCEA by Western blot. The humoral responses to CEA were predominantly IgG1 autoantibodies, with a minority of patients also demonstrating IgM autoantibodies.

The observation that CEA antibodies detected in our patients were of low titer and low avidity is supported by data from competitive inhibition assays (Table 1). Several considerations relevant to all circulating tumor-associated self-antigens may explain these results. First, the inability to effectively boost with rV-CEA due to viral neutralizing immune responses limits anti-CEA affinity maturation and lymphocyte expansion. Secondly, circulating antigen (i.e., CEA) defeats affinity maturation by removing the selective pressure that favors high avidity antibodies. Third, self-proteins like CEA are inherently poorly immunogenic. Finally, any high avidity antibodies that are produced are likely to be cleared from the circulation as immune complexes with soluble CEA or through binding to solid-phase CEA in normal or neoplastic tissues. The autoantibody that does

**Table 2** IgG subclass of patient serum CEA antibodies

<table>
<thead>
<tr>
<th>Subclass</th>
<th>UAB 9619</th>
<th>UAB 9501</th>
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<tbody>
<tr>
<td></td>
<td>Pt 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Pt 3</td>
</tr>
<tr>
<td>IgG1</td>
<td>3333&lt;sup&gt;b&lt;/sup&gt;</td>
<td>840</td>
</tr>
<tr>
<td>IgG2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgG3</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>IgG4</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
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<sup>a</sup> Pt, patient.

<sup>b</sup> Values shown (ng/ml) are the values of each IgG subclass bound to the CEA-coated plate for patient sera 5-8 weeks after immunization. Values greater than 38 ng/ml represent a positive result.

![Fig. 5 Binding of polyclonal monkey anti-CEA sera and COL-1 monoclonal anti-CEA antibody to solid-phase human CEA in the presence or absence of soluble human CEA.](image-url)
circulate is therefore likely to be of relatively low affinity and exist in equilibrium between free antibody and immune complexes with circulating antigen. Thus, the question arises as to what extent elevated serum CEA levels frequently encountered in patients with advanced adenocarcinoma adversely affect the ability to detect circulating anti-CEA autoantibodies. We examined this issue by adding varying concentrations of human CEA protein to relatively low titer, low avidity, polyclonal monkey anti-CEA sera selected to approximate patient anti-CEA sera. Significant inhibition of antibody detection was only observed at extremely high serum CEA concentrations not encountered in patients, i.e., greater than 10,000 ng/ml. In contrast, serum CEA concentrations occasionally encountered in the clinic (i.e., approximately 1,000 ng/ml) significantly inhibited ELISA detection of an anti-CEA monoclonal antibody. Thus, whereas clinically relevant concentrations of serum CEA can competitively inhibit binding of a high affinity monoclonal antibody to solid-phase CEA, inhibition of relatively low affinity polyclonal antisera only occurs at enormous serum CEA concentrations not seen in patients. This conclusion is also supported by the fact that competitive inhibition of patient anti-CEA sera binding to solid-phase CEA was observed with soluble CEA at 3 × 10^9 ng/ml sera but not at 3,000 ng/ml sera.

Another long-debated issue raised by the induction of anti-CEA autoantibodies is the potential influence of such antibodies on the ability to use serum CEA levels as a clinical indicator of disease progression or response (26). The CEA antibodies induced in our patients did not affect quantitation of exogenous human CEA added to these sera, suggesting that this may not be a major issue.

As reported previously, no evidence of autoimmune toxicity was observed among patients vaccinated with rV-CEA in these trials, despite the production of circulating autoantibodies (16). The low titer, low avidity, anti-CEA antibodies induced in the pilot Phase I/II trials reported here would likely have modest biological activity. Also, the incidence of anti-CEA antibody responses was low (7 of 32 patients). Thus, additional Phase I/II trials of rV-CEA priming followed by alternative booster immunization strategies with recombinant canarypox, plasmid DNA, or recombinant protein are warranted in an effort to increase the frequency and avidity of anti-CEA antibody responses as well as CEA-specific T-cell responses before Phase III trials.

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REFERENCES


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