Immunogenicity and Safety of a Recombinant Vaccinia Virus Vaccine Expressing the Carcinoembryonic Antigen Gene in a Nonhuman Primate

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ABSTRACT

We have previously reported the development of a recombinant vaccinia virus vaccine expressing the human carcinoembryonic antigen (CEA) gene, designated rV(NYC)-CEA. This construct has been shown to elicit specific anti-CEA immune responses and an antitumor effect in a murine tumor model. In the studies reported here, the safety and immunogenicity of this recombinant vaccinia virus were evaluated in a rhesus monkey model.

Human CEA is a Mr. 180,000 glycoprotein expressed in approximately 90% of gastrointestinal carcinomas and in some breast and non-small cell lung carcinomas. This family also includes normal cross-reacting antigen (NCA). Rhesus monkeys, like humans, have some NCA on the surface of their granulocytes. Eight monkeys were immunized 3 or 4 times by skin scarification with the recombinant CEA vaccine and four monkeys received wild-type vaccinia virus as control.

After three vaccinations, all rV(NYC)-CEA-vaccinated animals exhibited a strong anti-CEA antibody response as measured by enzyme-linked immunosorbent assay. The functional ability of these antibodies to mediate lysis of a CEA-bearing tumor cell was demonstrated using human effector cells. This response could be enhanced by interleukin 2. Cellular immunity to CEA was measured by delayed-type hypersensitivity upon intradermal challenge with purified CEA. Only those animals receiving the recombinant vaccine displayed significant anti-CEA responses. Furthermore, peripheral blood mononuclear cells from immunized monkeys were found to proliferate in response to CEA stimulation. All vaccinated monkeys developed local skin irritation at the site of the vaccination, regional lymphadenopathy, and low-grade fevers after immunization. Following immunization with rV(NYC)-CEA, the response was consistent with the usual constitutional symptoms seen with human smallpox virus immunization. Blood counts, differentials, and hepatic and renal chemistries remained normal in all animals throughout the study and for up to 1 year following the primary vaccination. No evidence of immunological cross-reactivity to NCA was found by either a fall in the granulocyte count or analyses for anti-NCA antibodies. Thus, the rV(NYC)-CEA vaccine appears to be safe in rhesus monkeys. The administration of a CEA recombinant vaccine to rhesus monkeys induces both a humoral and a cell-mediated immune response directed against human CEA.

INTRODUCTION

Protocols to induce active immunotherapy responses in cancer patients are currently being carried out using a wide range of immunogens, i.e., vaccines. Most of the studies to date have involved melanoma patients in which tumor cell extracts (1, 2) or oncolysates (3, 4) of tumor cell extracts have been used as immunogens. Few studies have been carried out to induce active immunotherapy responses in carcinoma patients. One of these studies has involved the use of X-irradiated allogeneic tumor cells obtained at surgery in combination with BCG as immunogen (5). At this time, the nature of the actual immunogens in these cellular or cell extract preparations has not been determined.

In recent years, gene products associated with human tumors have been identified. Identification of these gene products may form the basis for their use to induce specific active immunotherapy responses in cancer patients. The cloning of these tumor-associated genes now permits their insertion into attenuated viruses for use in recombinant vaccines.

A potential target for specific active immunotherapy of human carcinoma is CEA (6). The CEA gene has recently been cloned and characterized (7). CEA is a Mr. 180,000 glycoprotein which has been shown to be expressed in the vast majority of human colorectal, gastric, and pancreatic cancers, as well as in breast carcinomas and non-small cell lung carcinomas (8). Weak expression of CEA has also been observed in some normal colonic epithelial cells, and strong expression has been observed in fetal gut tissues. The CEA gene has been shown (8, 9) to belong to the immunoglobulin gene superfamily and CEA has been shown to share some homology with proteins expressed on some normal adult tissues, such as NCA found on normal human granulocytes, biliary glycoprotein, and pregnancy-specific glycoprotein. It is not known at this time whether CEA is immunogenic in normal individuals or cancer patients. Some reports (10–12) have indicated that immune complexes containing CEA and human IgM or IgG exist in the sera of some cancer patients. However, other reports (13–15) have indicated that the dissociated immunoglobulins from these complexes are not specific for CEA.

Vaccinia virus is a member of the pox virus family that has been used successfully as a live vaccine for the eradication of smallpox (16). Recently, a recombinant vaccinia virus expressing the human immunodeficiency virus Mr, 160,000 envelope glycoprotein has been evaluated in healthy subjects for safety and efficacy in inducing anti-human immunodeficiency virus immune responses (17). Recombinant vaccinia viruses containing the p97 melanoma-associated antigen (18, 19), the c-erbB-2 oncoprotein (20), and the human milk fat globule antigen (21) have also been constructed. The recombinant p97 melanoma construct is in early clinical trials. Recombinant vaccinia vaccines are being considered for use in cancer and infectious diseases because it has been shown in animal models that the co-presentation of a potential immunogen with highly immunogenic vaccinia proteins can elicit a strong immune response against the inserted gene products. Thus, for a putative weak immunogen such as CEA, a recombinant CEA vaccine merits consideration as a potential vaccine to induce specific active...
immunotherapy responses in carcinoma patients bearing CEA-expressing tumors.

We have recently constructed a recombinant vaccinia-CEA construct using the attenuated New York City strain of vaccinia virus (22); the recombinant has been designated rV(NYC)-CEA. The ability of the rV(NYC)-CEA construct to induce antitumor immunity was evaluated in a murine model in which the human CEA gene was transduced into murine colon carcinoma cells (23). This model was developed because CEA cannot be detected in rodent tumors using a series of anti-CEA MAbs. Vaccination of mice with the rV(NYC)-CEA vaccine rendered them resistant to growth of subsequently transplanted CEA-expressing tumors (22). Moreover, when mice bearing established CEA-transduced murine carcinomas were treated with the rV(NYC)-CEA tumors they showed either greatly reduced growth or complete regression. No toxicity was observed in any of the mice (22). Moreover, the rV(NYC)-CEA recombinant vaccine was shown to induce strong anti-CEA antibody responses as well as specific T-cell responses in mice (22). No CEA-specific immune responses or antitumor effects were observed when mice were administered the control vaccinia virus without the inserted CEA gene (designated V-NYC).

As a step towards a more clinically relevant model, we have now conducted studies to determine if the rV(NYC)-CEA vaccine can elicit specific active immune responses to CEA in a nonhuman primate. These studies were also conducted to help determine the safety associated with the use of this vaccine in a primate model.

MATERIALS AND METHODS

Vaccines. Control animals received the New York City Board of Health strain of vaccinia virus, obtained from the American Type Culture Collection (ATCC VR-325; Rockville, MD). The virus was grown in HeLa cells and purified by sucrose density gradient centrifugation (24). This virus was designated V-NYC. The recombinant vaccinia virus, expressing human CEA, was developed using the New York City Board of Health strain as described and characterized previously (22). It was also grown in HeLa cells and purified by sucrose density gradient centrifugation (24). This virus was designated rV(NYC)-CEA.

Immunization of Monkeys. Twelve adult male rhesus monkeys (Macaca mulatta), ages 5 to 7 years, were used and assigned to two vaccination groups of six animals each. Two immunization schedules were investigated. In experiment I animals were immunized 4 times on days 1, 42, 84, and 174 and in experiment II animals were vaccinated on days 1, 42, and 84. Doses of either 1 x 10^5 or 5 x 10^5 PFU of either rV(NYC)-CEA or V-NYC were administered by skin scarification. Two animals of each group received a high (5 x 10^5 PFU) or a low (1 x 10^5 PFU) dose of recombinant vaccine and two animals received 5 x 10^5 PFU of V-NYC as controls. The animals were housed and maintained in accordance with the guidelines of the National Cancer Institute Animal Care and Use Committee and the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services Publication NIH 85–23, revised 1985 by the FDA Center for Biologics Evaluation and Research Office of Biological Product Review, Division of Product Quality Control, Pathology and Primatology Laboratory, Bethesda, MD).

Antibodies. MAb COL-1 has previously been shown to react with human CEA (25, 26). MAb B6.2 has been shown to react with human NCA (27). MAbs B1.1 and COL-4 have been shown to react with epitopes shared by NCA and CEA (26, 28). MOPC-21 and UPC-10 (Litton Bionetics, Inc., Charleston, SC) were used as isotype-matched control MAbs.

Measurement of Antibody Titters. Anti-CEA antibody was quantitated by an ELISA. Microtiter plates were coated with 100 ng of purified CEA (International Enzyme, San Diego, CA), NCA [purified from crude perchloric acid extracts of normal human lung (29); a gift from M. Kuroki, Department of Biochemistry, School of Medicine, Fukuoka University, Fukuoka, Japan], or ovalbumin (Sigma, St. Louis, MO) in PBS. The plates were blocked with 5% BSA in PBS, dried, and stored at -20°C until used. The plates were incubated with various dilutions of monkey serum, as well as MAb COL-1 as a standard control, for 1 h at 37°C. Plates were washed, and antibody was detected with horseradish peroxidase-conjugated goat anti-human IgG Fe-specific antiserum (1:80000) (Southern Biotechnology Inc., Birmingham, AL) followed by a 10-min incubation with 100 μl of 2.8 nm o-phenylenediamine dihydrochloride in 0.015% hydrogen peroxide in 0.17 phosphate-citrate buffer, pH 5.0. The reactions were stopped by the addition of 25 μl of 4 N sulfuric acid and the absorbance was read at 490 nm using a Bio-Tek EL310 microplate ELISA reader (Winooski, VT). Serum titers to CEA were calculated using an optical density of 0.4. In the development of the ELISA for use with the monkey sera, three sources of goat anti-human IgG were used and only one source was found (Southern Biotechnology, Inc.) to detect monkey IgG.

Western Blot Analyses. Monkey antiserum obtained 35 days following the first inoculation of rV(NYC)-CEA was tested in a Western blot for reactivity to various antigens (30). To this end, CEA, ovalbumin, and NCA (each at 10 μg/lane) were dissolved in SDS-polyacrylamide gel electrophoresis sample buffer (0.125% Tris- HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol), boiled for 5 min, and subjected to electrophoresis using 5–20% linear gradient SDS-polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to nitrocellulose paper (0.45-μm pore size) at 4°C overnight at 30 V in transfer buffer (25 mm Tris-HCl, pH 8.3, 192 mm glycine, 20% methanol). The blots were then incubated in PBS containing 5% BSA for 1 h at room temperature and were washed with PBS containing 0.05% Tween 20. Ten ml of a 1:200 dilution of antiserum in PBS with 1% BSA were added to each nitrocellulose strip and incubated for 2 h at room temperature with gentle agitation. After washing with PBS containing 0.05% Tween 20, the blots were incubated for 1 h at room temperature with 5 x 10^5 cpm/ml ^125I-labeled goat anti-human IgG (heavy plus light chain). The filters were washed extensively overnight and exposed to Kodak XAR X-ray film with an intensifying screen at -70°C for 18 h. Preimmunization serum was utilized as a negative control.

ADCC. Human effector cells (PBMCs) were obtained from heparinized blood drawn from healthy human adult donors. Cells were obtained by density gradient centrifugation in lymphocyte separation medium (Organon Teknika, Durham, NC) (31). Effector cells and target cells were assayed in the absence and presence of preimmune monkey serum or antiserum derived 21 days following the second vaccination. Target cells were the MC-38 (non-CEA-expressing) or the CEA-transduced MC-38-CEA-2 (human CEA-expressing) murine colon adenocarcinoma cell lines (23). These cells were labeled with 200 μCi of indium-111 oxide (1 mCi/ml; Amersham, Arlington Heights, IL) in RPMI 1640 with 10% fetal calf serum for 5 min at room temperature. The target cells (1 x 10^5) were added to 96-well sterile U-bottomed microtiter plates (Costar, Cambridge, MA) and were incubated with serum (diluted 1:50 and 1:250) for 1 h. Human effector cells were added and the plates were incubated for 18 h at 37°C in a humidified atmosphere containing 7% CO2. Experiments were carried out in triplicate. Specific lysis was calculated using the formula:

% lysis = \frac{\text{experimental release (cpm)} - \text{spontaneous release (cpm)}}{\text{total release (cpm)} - \text{spontaneous release (cpm)}}

In a separate experiment, the effector cells were pretreated for 18 h with 100 units/ml human recombinant IL-2 (Cetus, Emeryville, CA) in RPMI 1640 supplemented with 2% glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal calf serum for 24 h at 37°C in 7% CO2. After incubation, nonadherent cells were harvested, washed 3 times in complete medium, and tested for ADCC activity.
Lymphoproliferative Assay. Monkey PBMC from heparinized blood, 6 weeks to 12 months after the last vaccination, were isolated on lymphocyte separation medium (Organon). PBMCs were cultured by plating 2 × 10^6 cells/well in 0.2 ml of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum in flat-bottomed 96-well plates (Costar) for 6 days with the appropriate antigens or for 3 days with concanavalin A (Sigma). Cells were labeled for the final 18–24 h of the incubation with 1 μCi/well [3H]thymidine (New England Nuclear, Wilmington, DE) and were harvested with a PHD cell harvester (Cambridge Technology, Cambridge, MA). The incorporated radioactivity was measured by liquid scintillation counting (LS 3801; Beckman, Duarte, CA). The results from triplicate wells were averaged and are reported as mean ± SEM.

DTH Testing. DTH responses were assayed by skin testing 7 days following the last immunization. Purified CEA (Vitro Diagnostics, Littleton, CO) and ovalbumin (Sigma) at 100 μg in 0.1 ml of PBS were injected intradermally. As a positive control, 1 × 10^7 PFU of UV light-inactivated (254 nm for 10 min) vaccinia virus were also injected. Swelling and erythema were measured 48 h later with a Mitutoyu Digimatic micrometer (Mitutoyu Measuring Instruments, Maplewood, NJ). Results were expressed in mm, with 1 mm being equivalent to 0.0254 mm. Punch biopsies were taken of positive responses and the DTH nature of the reaction was confirmed by histopathological examination.

Toxicology. Physical examinations were performed on ketamine (Keta Set, ketamine hydrochloride, 10 mg/kg; Fort Dodge Laboratories, Inc., Fort Dodge, IA)-sedated animals. Rectal temperatures and weights were recorded for each monkey on a weekly basis. The vaccination site was observed and erythema and swelling were measured with a caliper. Each animal was examined for regional lymphadenopathy, hepatomegaly, and splenomegaly. Any other gross abnormalities were also recorded.

Blood was obtained by venipuncture from the femoral vein of ketamine-sedated animals before and after each immunization. A complete blood count and differential were performed by METPATH (Mid Atlantic Regional Laboratory, Rockville, MD). Serum chemistries to evaluate hepatic and renal function were also performed by METPATH. Results were compared to normal primate values.

RESULTS

CEA and NCA Expression in Rhesus Monkeys. NCA, but not CEA, has been previously shown to be expressed on human granulocytes. To evaluate the appropriateness of the rhesus monkey as a model for the ability of the rV(NYC)-CEA vaccine to elicit specific immune responses and to evaluate its safety, monkey granulocytes were analyzed for NCA and CEA expression using specific MAbs. Monkey and human granulocytes isolated from the peripheral blood were analyzed for surface NCA and CEA expression by flow cytometry (32). Table 1 summarizes the binding of anti-NCA and anti-CEA MAbs to granulocytes isolated from five different monkeys and two separate human samples. NCA expression was measured by the binding of MAbs B1.1, B6.2, and COL-4, which recognize three different epitopes on human granulocyte NCA (28). CEA expression was measured by COL-1 binding. Granulocytes from all five monkeys reacted with B1.1 and B6.2. Fig. 1 shows a representative flow cytometric analysis of B1.1 reactivity with monkey and human granulocytes. The percentage of B1.1-positive cells ranged from 61% to >80%, whereas the percentage of monkey granulocytes reactive with B6.2 was, in general, considerably lower. The range of B6.2-reactive granulocytes from four of the five monkeys was 8.8–16.7%. Granulocytes from a single monkey expressed high surface NCA (i.e., 96.1%), as measured by B6.2 binding. COL-4 was reactive with the granulocytes isolated from the only monkey tested. The anti-CEA MAb COL-1 did not react with any of the monkey or human granulocyte preparations. The flow cytometric data indicate that MAbs that are capable of recognizing three different NCA epitopes on the surface of human granulocytes also react with similar epitopes expressed by monkey granulocytes. The levels of expression of those B1.1 (Fig. 1), B6.2, and COL-4 NCA epitopes on the monkey granulocyte cell surface are, however, much lower than those measured on human granulocytes. The rhesus monkey, in any case, appears to represent a model for analyzing the potential consequences of anti-NCA immune responses elicited following the administration of the rV(NYC)-CEA vaccine.

Experimental Design. Table 2 delineates the protocol used in the immunization of 12 rhesus monkeys with either rV-(NYC)-CEA or control V-NYC vaccines by skin scarification. In the first experiment, two monkeys (1 and 2) were administered V-NYC at 5 × 10^8 PFU/monkey on day 1, with boosts of V-NYC being given at days 42, 84, and 174. Monkeys 3 and 4 received the same dose and schedule of inoculum except that two boosts were given (on days 42 and 84) compared to three boosts in experiment I. These doses were chosen to ascertain the maximum humoral and cell-mediated responses to CEA. The maximum tolerated dose for safety and toxicity as well as to obtain maximum humoral and cell-mediated responses to CEA.

Physical Consequence of Immunization. The area of the lesions induced by the vaccines was analyzed 24 h following each

Table 1 Comparison of anti-NCA and anti-CEA MAb reactivity with monkey and human granulocytes

<table>
<thead>
<tr>
<th>Surface granulocyte MAb reactivity</th>
<th>Monkey</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAb</td>
<td>MAb*</td>
</tr>
<tr>
<td>Antigen</td>
<td>No. of</td>
<td>No. of</td>
</tr>
<tr>
<td></td>
<td>samples</td>
<td>samples</td>
</tr>
<tr>
<td>B1.1</td>
<td>NCA/CEA</td>
<td>5/5</td>
</tr>
<tr>
<td>B6.2</td>
<td>NCA</td>
<td>5/5</td>
</tr>
<tr>
<td>COL-4</td>
<td>NCA/CEA</td>
<td>1/1</td>
</tr>
<tr>
<td>COL-1</td>
<td>CEA</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* Values represent the range of cells which were positive for the binding of each of the listed MAbs. In all experiments, isotype-matched, control antibodies MOPC-21 (IgG1) and UP:10 (IgG2a) were included and the percentage of monkey and human granulocytes reactive with each of those MAbs was <4.0%.
inoculation. In general, more swelling was seen after the first two inoculations, compared to the third or fourth inoculation (Fig. 2A). The duration of the lesions following each immunization, however, was approximately the same (Fig. 2B). Regional lymph node swelling following vaccination was greater in some monkeys following the first immunization, compared to the second, third, or fourth immunization (Fig. 2C). In general, no differences were seen in the aforementioned parameters with the use of the rV(NYC)-CEA or V-NYC vaccines.

**Humoral Responses.** As indicated previously, monkeys 3 to 6 and 9 to 12 were administered rV(NYC)-CEA while monkeys 1, 2, 7, and 8 were administered control V-NYC. Sera from each of these monkeys were analyzed by ELISA for immunoreactivity to CEA, NCA, and ovalbumin as a control antigen. As seen in Table 3, all preimmune sera were negative against all three antigens. At day 28 following primary immunization, strong (greater than 1:1000 serum dilution) antibody titers were observed to CEA in two of the eight rV(NYC)-CEA-inoculated monkeys. At day 49, 1 week following the first boost, antibody titers at or greater than a 1:250 serum dilution were seen in all eight monkeys inoculated with rV(NYC)-CEA and in none of the monkeys receiving V-NYC. Similar results were seen at day 63. At day 91 (7 days following the second boost) antibody titers of greater than 1:1000 serum dilution were seen in all seven monkeys tested that received rV(NYC)-CEA, with titers greater than or equal to 1:5800 in four monkeys. An immune response to ovalbumin was also seen in this monkey. Two other monkeys, one receiving rV(NYC)-CEA and one receiving V-NYC, also showed some antibody titer to NCA at day 91; however, identical titers were seen for the control ovalbumin antigen at the same time, thus indicating a potential nonspecific reactivity. It thus appears that rV(NYC)-CEA induced a strong immune response on CEA in rhesus monkeys, with little or no response to NCA-specific epitopes. The temporal nature of the anti-CEA immune response is depicted in Fig. 3.

A serum sample derived from one rhesus monkey 35 days following an initial vaccination with rV(NYC)-CEA was analyzed by Western blot methodology for reactivity to CEA, NCA, and a control protein, ovalbumin. As shown in Fig. 4, an antiserum recognized purified CEA (Fig. 4, lane 1) but not ovalbumin (Fig. 4, lane 2) or purified NCA (Fig. 4, lane 3). Preimmunization serum derived from the same monkey failed to detect either CEA, NCA, or ovalbumin (data not shown). Control monoclonal antibodies COL-1 and B6.2, which recognize CEA and NCA, respectively, were utilized as positive controls in this experiment (data not shown).

Biological activity of the immunoglobulins induced by the rV(NYC)-CEA vaccine was analyzed by ADCC. Sera from all of the recombinant vaccine-treated animals were tested using human PBMC as effectors and human CEA-transduced murine tumor cell lines as targets. Nontransduced cells were used as controls. A representative example of ADCC is seen in Fig. 5A, where specific lysis of the CEA-expressing tumor cells was seen.

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**Table 2** Inoculation protocol of rhesus monkeys with the CEA recombinant and wild-type vaccinia virus

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Monkey no.</th>
<th>Immunogen</th>
<th>Dose</th>
<th>Days of boosts</th>
</tr>
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<tbody>
<tr>
<td>I.</td>
<td>1</td>
<td>V-NYC</td>
<td>5 x 10^8</td>
<td>42, 84, 174</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>V-NYC</td>
<td>5 x 10^8</td>
<td>42, 84, 174</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>rV(NYC)-CEA</td>
<td>5 x 10^8</td>
<td>42, 84, 174</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>rV(NYC)-CEA</td>
<td>5 x 10^8</td>
<td>42, 84, 174</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>rV(NYC)-CEA</td>
<td>1 x 10^8</td>
<td>42, 84, 174</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>rV(NYC)-CEA</td>
<td>1 x 10^8</td>
<td>42, 84, 174</td>
</tr>
<tr>
<td>II.</td>
<td>7</td>
<td>V-NYC</td>
<td>5 x 10^8</td>
<td>42, 84</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>V-NYC</td>
<td>5 x 10^8</td>
<td>42, 84</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>rV(NYC)-CEA</td>
<td>5 x 10^8</td>
<td>42, 84</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>rV(NYC)-CEA</td>
<td>5 x 10^8</td>
<td>42, 84</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>rV(NYC)-CEA</td>
<td>1 x 10^8</td>
<td>42, 84</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>rV(NYC)-CEA</td>
<td>1 x 10^8</td>
<td>42, 84</td>
</tr>
</tbody>
</table>

**a** Dermal route doses at 6-week intervals.  
**b** Group I received four doses of immunogen.  
**c** Days after first inoculation (day 1).
Table 3 Primates antibody response to inoculation with recombinant vaccinia virus

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Immunogen</th>
<th>Day 28</th>
<th>Day 49</th>
<th>Day 63</th>
<th>Day 91</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V-NYC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>V-NYC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>400</td>
</tr>
<tr>
<td>3</td>
<td>rV(NYC)-CEA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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</tr>
<tr>
<td>6</td>
<td>rV(NYC)-CEA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Monkeys received vaccinations on days 1, 42, and 84. Sera were tested via ELISA (see "Materials and Methods").
b OVA, ovalbumin.
c ND, not detectable; limit of detection was 1:50 dilution.
d Animals bled before boosting.
e NT, not tested.

Fig. 3. Antibody responses to inoculation with recombinant vaccinia virus. Monkeys were vaccinated on days 1, 42, and 84 (arrowheads), as described in the text, with either V-NYC monkey 1 (open circles), monkey 2 (open squares) or rV-(NYC)-CEA monkey 3 (closed circles), monkey 5 (closed squares), monkey 6 (closed triangles). Anti-CEA antibody was quantitated at different time points by ELISA as described in "Materials and Methods."

Fig. 4. Western blot of purified CEA using a serum sample derived from one monkey vaccinated with rV-(NYC)-CEA. A rhesus monkey received one inoculation with 5 x 10⁹ PFU of rV-(NYC)-CEA. Thirty-five days following the inoculation, serum at a 1:200 dilution was tested in a Western blot against CEA (lane 1), ovalbumin (lane 2), and NCA (lane 3). Equal amounts of protein (10 µg) were added to each lane.

Cellular Responses. Monkeys were tested for DTH responses 7 days following their last inoculation. As seen in Fig. 6, all seven monkeys receiving rV(NYC)-CEA as immunogen responded with a positive DTH response to UV-inactivated V-NYC vaccinia virus. On the other hand, none of the 12 monkeys responded to the ovalbumin control antigen. None of the monkeys inoculated with V-NYC responded to CEA as challenge antigen while, in contrast, seven of eight monkeys immunized with rV(NYC)-CEA responded to the purified CEA preparation.

Responses in monkeys immunized with rV(NYC)-CEA or V-NYC were also analyzed using lymphoproliferative assays. PBMC were isolated from immunized monkeys 6 or 12 months after their final immunization. As seen in Table 4, all monkeys analyzed responded well, regardless of whether they received using serum from a monkey immunized with rV(NYC)-CEA while no lysis was observed using the nontransduced tumor cells as targets. This monkey had the highest antibody titer to CEA. However, the range of lysis in the other recombinant vaccine-treated animals was 10 to 50% (data not shown). Less than 5% lysis was seen with preimmune serum or serum of a monkey inoculated with V-NYC (data not shown). As shown in Fig. 5B, the ADCC activity of the serum from rV(NYC)-CEA-immunized monkeys was enhanced using IL-2-activated human PBMC.

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rV(NYC)-CEA or V-NYC, to the lymphocyte mitogen concanavalin A as well as to stimulation with UV-inactivated V-NYC. Little or no lymphocyte response specific for CEA compared to ovalbumin was seen in the monkeys immunized with V-NYC. Differential responses to CEA versus medium alone or ovalbumin, however, were seen in the three monkeys analyzed that were immunized with rV(NYC)-CEA, even at 12 months following the last immunization (Table 4). These results and the DTH results detailed above thus demonstrate the ability of the rV(NYC)-CEA vaccine to elicit cell-mediated immune responses to CEA.

Toxicology. Monkeys receiving the wild-type vaccinia virus were compared to monkeys receiving the recombinant vaccinia virus with respect to temperature, weight, regional lymphadenopathy, and the presence of splenomegaly and hepatomegaly. Mild temperature elevations were seen in all animals following vaccination (Table 5). A mild regional lymphadenopathy was observed for several weeks following the immunizations, as described in Fig. 2C. There was no evidence of weight loss, hepatomegaly, or splenomegaly in any of the animals, and there were no differences between control and recombinant vaccine-treated animals. Animals were tested for complete blood count, differential, and hepatic and renal chemistries. Complete blood counts remained within normal limits throughout the study in both recombinant virus-immunized and wild-type virus-immunized animals. Table 6 reports preimmune values and those obtained from samples taken 6 months to 1 year following the first immunization. Upon physical examination 1 year after vaccination, one of the rV(NYC)-CEA-vaccinated monkeys was observed to have an abdominal mass and was killed. Autopsy revealed an 1830-g right suprarenal mass. Microscopically, the tumor was consistent in appearance with an adrenocortical adenocarcinoma. The mitotic rate was <1 mitosis/10 high power fields, indicative of an extremely slowly growing tumor. The undescended testes of the 7-year-old male showed tubules filled with Sertoli cells. No spermatogenesis was found and interstitial cells were not prominent. The small bowel showed epithelial hyperplasia, goblet cell metaplasia, and mild to moderate inflammation of the lamina propria, which was not characteristic of any infectious autoimmune, neoplastic, or other pathologic process. The colon, rectum, stomach, kidney, and other tissues were histologically normal. This monkey had previously been administered an adenovirus vaccine and a respiratory syncytial virus vaccine, among other treatments. In light of the extremely low mitotic index of the tumor and its large size, it is believed that the tumor most likely arose years prior to the rV(NYC)-CEA administration.

All blood counts of all vaccinated monkeys were within normal range throughout the study. The differential count remained normal in all animals throughout the study period. Hepatic function was assessed by measuring serum albumin,
RECOMBINANT CEA VACCINE

Table 4 Lymphoproliferation responses by PBMC from rhesus monkeys immunized with V-NYC or rV(NYC)-CEA

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Months after final immunization</th>
<th>Immunogen*</th>
<th>[3H]Thymidine incorporation (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Medium</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>rV(NYC)-CEA</td>
<td>3,665 ± 291</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>rV(NYC)-CEA</td>
<td>1,833 ± 203</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>V-NYC</td>
<td>2,068 ± 221</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>V-NYC</td>
<td>3,807 ± 542</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>V-NYC</td>
<td>7,200 ± 654</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>V-NYC</td>
<td>5,189 ± 616</td>
</tr>
</tbody>
</table>

a Rhesus monkeys were inoculated with 5 × 10^6 PFU of rV(NYC)-CEA or V-NYC on three (monkeys 9, 7, and 8) or four (monkeys 3, 4, and 1) separate occasions separated by 6 weeks. PBMC were then isolated from immunized monkeys 6 months (monkeys 3, 9, 7, and 8) or 12 months (monkeys 4 and 1) after their final exposure.

b PBMC (2 × 10^6/well) were incubated without (medium control) or with various stimuli, i.e., concanavalin A (Con A) (2 μg/ml), UV-inactivated V-NYC (1 × 10^7 PFU/ml), purified CEA (100 μg/ml), or purified ovalbumin (100 μg/ml), for up to 3 days for concanavalin A or 6 days for other antigens. Data are reported as the mean ± SEM of triplicate wells.

d Differential blood count and ELISA. There was no difference in differential counts in any of the vaccinated animals (Table 6).

e No significant differences were found in these values between vaccinated and wild-type virus-vaccinated animals throughout the study. These values did not differ from normal ranges throughout the study. Differences between mean values before and after immunization are reported in Table 7.

Table 5 Mean rectal temperature and weight changes in rhesus monkeys receiving recombinant or wild-type vaccine

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Temperature (°C)</th>
<th>Weight (pounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before immunization</td>
<td>After immunization</td>
</tr>
<tr>
<td>V-NYC (n = 4)</td>
<td>38.5 ± 0.2</td>
<td>39.1 ± 0.1</td>
</tr>
<tr>
<td>rV(NYC)-CEA (n = 8)</td>
<td>38.1 ± 0.1</td>
<td>39.1 ± 0.1</td>
</tr>
</tbody>
</table>

a Normal rhesus monkey temperature is 37-38°C.
b Mean ± SEM.

c Serum glutamic pyruvic transaminase.
d γ-glutamyl transpeptidase.
e Blood urea nitrogen.

b 6 months to 1 year following primary vaccination.
c Serum glutamic oxaloacetic transaminase.

We have constructed a recombinant vaccinia virus expressing human CEA and have recently shown its antitumor efficacy for murine tumors expressing human CEA (22, 33). These effects could be correlated with humoral and cellular proliferative immune responses to CEA in these animals. This study was conducted to establish further the immunogenicity of this recombinant vaccine in a more clinically relevant model. No colon tumor model exists in nonhuman primates. In this paper we have demonstrated both humoral and cell-mediated immune responses directed against human CEA in primates. The recombinant vaccine construct was able to elicit an anti-CEA antibody response in all recombinant vaccinated animals, and this response could be boosted by repeated vaccinations. While it is unknown at this time whether more than three vaccinations
would be optimal, it is clear from this study that three vaccinations resulted in an elevated antibody titer in all animals; this response was specific for CEA, in that no specific antibody response was detected in control NYC vaccinia virus-treated animals. In addition, this antibody is capable of eliciting a specific ADCC reactivity, lysing only those tumor cells expressing CEA.

The importance of cell-mediated immunity in tumor rejection has been suggested (34). To investigate these immunological mechanisms, we performed lymphoproliferative assays using PBMC from animals receiving the recombinant vaccine. The results of these assays suggest that only PBMCs from monkeys receiving recombinant vaccine are capable of proliferation in response to CEA antigen. Further evidence of cell-mediated immunity was demonstrated by a positive DTH response to CEA in recombinant vaccine-inoculated animals. These findings suggest that the recombinant vaccine may elicit the proper immune mechanisms responsible for tumor rejection.

Although these responses can be elicited in monkeys, it is uncertain whether these same responses can be induced in humans. CEA is an oncotelic tumor antigen. It is expressed extensively in fetal colon, in gastrointestinal tract adenocarcinoma, and in certain benign conditions. The immunogenicity of CEA in these conditions has not been extensively studied. As mentioned previously, there are conflicting reports as to whether anti-CEA immune complexes exist in cancer patients (10–15). One possibility is that CEA may be only weakly immunogenic and the host immune response may be compensated for by the co-presentation of CEA with live vaccinia virus. Thus, this recombinant CEA vaccine merits consideration as a potential source for the induction of specific active immunotherapy responses in patients bearing CEA-expressing tumors.

Before this vaccine can be considered for clinical use, specific safety questions should be addressed. In general, vaccinia virus is a weak human pathogen (35). Following vaccination, local erythema, induration, low-grade fever, and regional lymphadenopathy are common. The virus replicates in the epidermal cells of the skin and the virus is usually cleared within 14 days. Major complications such as generalized vaccinia infection can occur in active eczema and in immunocompromised patients. All monkeys, whether given r(NYC)-CEA or V-NYC, exhibited the usual low-grade constitutional symptoms of a viral infection. There was no evidence of any other adverse reactions, as indicated by normal blood counts, differentials, and hepatic and renal chemistries. The monkeys appeared healthy, without any physical signs of toxicity, throughout the study.

CEA shows 65–75% amino acid sequence homology with NCA (8). NCA is present on the surface of human granulocytes, and a molecule immunologically related to NCA is present on the surface of rhesus granulocytes (Table 1). The possibility thus exists of eliciting an immune response against NCA epitopes with the recombinant vaccinia-CEA vaccine. However, after several vaccinations granulocyte counts remained normal and no evidence of anti-NCA antibodies was detected in vaccinated animals. These responses remained normal up to 12 months following primary vaccination. However, it is possible that low levels of anti-NCA antibodies were generated but could not be detected by the assays used. The safety and ability to elicit specific anti-CEA humoral and cell-mediated immune responses of the recombinant CEA vaccinia vaccine have thus now been demonstrated in rodents and in nonhuman primates.

REFERENCES

26. Kuroki, M., Greiner, J. W., Simpson, J. F., Primus, F. J., Guadagni, F., and...


Immunogenicity and Safety of a Recombinant Vaccinia Virus Vaccine Expressing the Carcinoembryonic Antigen Gene in a Nonhuman Primate

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