Baculovirus Recombinant Expressing a Secreted Form of a Transmembrane Carcinoma-associated Antigen

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ABSTRACT

GA733-2 is a monoclonal antibody-defined, 40-kDa glycoprotein antigen that is associated with carcinomas of various origins. Hydrophobicity analysis of the protein sequence predicted by complementary DNA (cDNA) has suggested that the GA733-2 antigen is a type I membrane protein. In this study, the polymerase chain reaction was used in a strategy to omit cDNA sequences for the transmembrane and cytoplasmic domains, thereby converting the extracellular domain into a secretory strategy to omit cDNA sequences for the transmembrane and cytoplasmic domains, thereby converting the extracellular domain into a secretory protein. Full-length and truncated cDNAs were cloned into the baculovirus transfer vector pVL1392 and introduced into Autographa californica nuclear polyhedrosis virus by homologous recombination. The full-length cDNA baculovirus recombinant directed the expression of a 40-kDa glycoprotein that was confined to infected Spodoptera frugiperda cells, whereas cells infected with the truncated cDNA baculovirus recombinant abundantly secreted a 31-kDa glycoprotein into the culture medium. Reombinant secretory antigen displayed an in vitro immunoreactivity to monoclonal antibody and an in vivo immunogenicity in mice that were similar to native antigen. The facile purification of mg quantities of carcinoma-associated antigen will enable an evaluation of its immunogenicity in cancer patients.

INTRODUCTION

The GA733-2 antigen is a 40-kDa human cell surface glycoprotein defined by the murine mAbGA733 (1). This antigen has been found to be associated with a variety of human carcinomas such as colorectal, pancreatic, and breast carcinoma (2, 3). The independently derived mAbs GA733, CO17-1A (4, 5), M77, M79 (3), 323/A3 (6), among others, all define the GA733-2 antigen. mAbs that define tumor cell surface antigens are being evaluated for the diagnosis and immunotherapy of cancer. Initial studies of mAbs CO17-1A and GA733 have demonstrated both cytotoxic effects in vitro and tumoricidal in vivo responses in experimental animal models (2, 4). Clinical trials are under way and have shown strong mAb tumor binding (7) and cases of partial and complete regression of disseminated cancer (8, 9). Since only mg quantities of the native antigen are available, therapeutic approaches are limited to passive immunization with mAb and active immunization with anti-idiotypic mAb (Ab2).

Molecular clones for the GA733-2 antigen have been isolated by immunoselection of monkey COS cells transfected with a cDNA expression library derived from a human colon carcinoma cell line (10). The GA733-2 sequence is identical to independently isolated cDNAs encoding the adenocarcinoma-associated antigen (11, 12) and the epithelial glycoprotein antigen (13). The GA733-2 coding region is 54% identical to the GA733-1 gene, a retroposon that is abundantly transcribed in pancreatic carcinoma cell lines (14). The GA733-2 chromosomal gene contains exons encoding an epidermal growth factor-like repeat and a thyroglobulin type I repeat (4); however, the biological function of the GA733-2 antigen remains unknown. Based on hydrophobicity analysis of the predicted antigen sequence, GA733-2 resembles a type I membrane protein. An amino-terminal 23-residue signal peptide is followed by a 242-residue extracellular domain containing 12 cysteine residues and 3 potential N-glycosylation loci, a 23-residue transmembrane domain, and a highly charged 26-residue intracellular anchor (10).

To allow for immunological, physical, and biochemical studies of the GA733-2 antigen, recombinant antigen will be required. The purification of type I membrane proteins can be facilitated by altering their DNA sequences to direct the synthesis of secreted protein. Membrane proteins which have been manipulated in this manner are the hemagglutinin of influenza virus (15), the glycoprotein of vesicular stomatitis virus (16), myelin-associated glycoprotein (17, 18), and CD4 (19).

The baculovirus-insect cell expression system has been well recognized for its ability to abundantly express recombinant proteins, which most often resemble native protein with respect to function, immunoreactivity, and immunogenicity (20). Baculovirus has been exploited for production of a variety of enzymes, transmembrane proteins, and secretory proteins such as tissue plasminogen activator, interleukin 2, and human β-interferon (reviewed in Ref. 20). In this study, two different GA733-2 baculovirus recombinants were constructed and expressed. One contained the full-length version of GA733-2 (GA733-2F), derived from restriction enzyme digestion of the CD8 recombinant plasmid (10). The other consisted of a PCR-synthesized, truncated version of the cDNA (GA733-2E) in an attempt to achieve secretion of the GA733-2 antigen into the culture medium.

MATERIALS AND METHODS

PCR Synthesis of GA733-2 Extracellular Domain. Oligonucleotide primers were synthesized by automated phosphoramidite chemistry on a model 380A DNA synthesizer (Applied Biosystems). PCR synthesis was performed with GA733-2 cDNA template (1 ng) (10), oligonucleotide primers (1 μM each), and Thermus aquaticus polymerase in a volume of 100 μl. PCR was carried out for 35 cycles (denaturing 94°C for 1 min, annealing 40°C for 2 min, elongation 72°C for 3 min) by using the DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT). The final elongation step was extended by 7 min. The reaction was extracted with phenol/chloroform and the DNA was purified by glass bead adsorption (Bio 101, Inc., La Jolla, CA). PCR products were analyzed in ethidium bromide-stained agarose gels (2% Nusieve-1% Seakem; FMC BioProducts, Rockland, ME).

Construction of Baculovirus Transfer Vectors pGA733-2F and pGA733-2E. Optimal conditions for the ligation of GA733-2F [XbaI restriction enzyme-cleaved insert of CD8 recombinant, described

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PCR ENGINEERED CARCINOMA-ASSOCIATED ANTIGEN

earlier (10) to XbaI-digested pVL1392 [Invitrogen Corp., San Diego, CA] with T4 ligase [Boehringer-Mannheim, Mannheim, Germany] were an insert:vector molar ratio of 3:1 and a total concentration of 5' ends of 0.4 μM [4.36 μg DNA/10 μl]. Ligated products were transformed into competent DH5α Escherichia coli cells, and insert-containing clones were identified by bacterial colony hybridization (21). The orientation of the pGA733-2F cDNA was established by restriction mapping.

GA733-2E PCR product was digested with PstI and XbaI; directional cloning into pVL1392 was carried out by using an insert:vector molar ratio of 3:1 and a total concentration of 5' ends of 0.03 μM (390 ng DNA/10 μl). Recombinant pGA733-2E was isolated and the insert was sequenced to monitor the fidelity of the PCR synthesis.

Generation of Baculovirus Recombinants. Sf9 Spodoptera frugiperda insect cells (Invitrogen Corp.) were cultured at 27°C in supplemented Grace's Insect Medium (Gibco, Gaithersburg, MD) containing 10% fetal bovine serum and antibiotics. An aliquot of 1.5 × 10⁴ cells was transfectioned with 2 μg of CsCl-purified pGA733-2F or pGA733-2E, respectively, and 1 μg of purified AcNPV viral DNA, by using the calcium chloride precipitation method (22). After 7 days of incubation a viral stock containing wild-type and recombinant viruses was harvested.

Recombinants were enriched by two rounds of dot-blot hybridization screening (23). Fifty μl of serially diluted viral supernatant (10⁻²–10⁻⁴, first round; 10⁻³–10⁻⁵, second round) were used to infect 1.5 × 10⁴ cells in each well of a 96-well plate. Supernatants were collected 6 days p.i. Cells were lysed in 0.2 M NaOH, vacuum blotted onto nitrocellulose filters (Schleicher & Schuell, Keene, NH), and hybridized to a nick-translated, ³²P-labeled GA733-2 cDNA insert probe (1 × 10⁶ counts/ml). Supernatants containing recombinant virus were indicated by autoradiography of the filters.

Plaque purification was carried out by 1–2 rounds of visual screening of infected Sf9 cell monolayers with 0.75% agarose overlay (Seakem). Occulsion body-negative plaques, representing pure recombinant viral colonies, were picked 7 days p.i. Agarose plugs were eluted in culture medium that was used to infect 2 × 10⁶ Sf9 cell monolayers in 25-ml culture flasks, yielding 5 ml of pure high-titer viral stock supernatants (2–5 × 10⁶ plaque-forming units/ml).

Expression of Recombinant Antigens. Aliquots of 2 × 10⁶ Sf9 cells in midlogarithmic growth phase (99% viable) were infected with pGA733-2F and pGA733-2E viral recombinants including a wild-type AcNPV virus control at a multiplicity of infection of at least 10. In the baculovirus life cycle, protein production regularly reaches a maximum 48 to 72 h p.i. Recombinant protein was therefore harvested 64 h p.i. After the first 24 h of incubation in supplemented Grace's medium (TNM-FH medium), the cells were cultured for 40 h under serum-free conditions (SF900 medium; Gibco). Supernatants were collected and cell pellets were extracted with a buffer containing NP40 [0.5% NP40–0.14 M NaCl–0.01 M NaF–0.01 M Tris (pH 7.5)–5 mM EDTA–1 mM phenylmethysulfonyl fluoride–8 μg/ml aprotinin]. Sf9 cell lysates were clarified by centrifugation at 100,000 × g. Cell lysates were prepared from positive control, colorectal carcinoma SW948 cells in the same manner. 2 μg of protein (Lowry) from supernatants or detergent extracts were boiled for 90 s in nonreducing loading buffer [2% SDS–62.5 mm Tris–Cl (pH 6.8)–10% glycerol–0.001% bromophenol blue] prior to electrophoresis in a 15% SDS-polyacrylamide gel (24). Samples were electroblotted onto nitrocellulose filters (Bio-Rad, Richmond, CA) and reacted with the murine mAb GA733 (1 μg/ml). Antibody binding to the recombinant proteins was visualized through an alkaline phosphatase-conjugated anti-mouse IgG (Promega, Madison, WI).

N-Glycosylation Inhibition of Recombinant Antigens. An N-glycosylation inhibition study was carried out for recombinant GA733-2F antigen and GA733-2E antigen. Cells were seeded and infected as described above. After 24 h of incubation, TNM-FH medium was replaced by serum-free SF900 medium containing 2 or 5 μg/ml of the N-glycosylation inhibitor tunicamycin (25). After a total incubation time of 64 h, protein was harvested from the supernatant and from lysed cells as described earlier. Forty μg of protein were applied to each lane; electrophoresis and immunoblotting were carried out as described above.

Preparative Isolation of Native and Secreted Recombinant Antigen. SW1116 colorectal carcinoma cells have been described previously (26). Native GA733-2 antigen was obtained from NP40 lysate of SW1116 tumors grown in nude mice. Detergent extracts were purified on CNBr-activated Sepharose 4B columns (Sigma Chemical Co., St. Louis, MO) coupled with mAb GA733 as described previously (1).

For large-scale production of secreted recombinant antigen, Sf9 cells were infected with pGA733-2E recombinant baculovirus at a multiplicity of infection of 5. The cells were cultivated at 27°C in spinner flasks at 2 × 10⁶ cells/ml in TNM-FH medium for 24 h and then in serum-free SF900 medium for an additional 40 h. The infected Sf9 cell culture supernatants were harvested by centrifugation and passed over a mAb GA733 immunoaffinity column. Purity of the GA733-2E preparations was demonstrated by SDS-polyacrylamide gel electrophoresis and staining with silver nitrate.

Immunization of Mice. Female BALB/c mice, 16 weeks old (Harlan Sprague-Dawley, Indianapolis, IN), were immunized s.c. 3 times, at 2-week intervals, with 1 to 5 μg of aluminum hydroxide-precipitated (27) native GA733-2 antigen, secreted recombinant GA733-2E antigen, or BSA. Mouse sera were obtained before and at 24, 38, 50, and 70 days following the first immunization.

Antibody Binding Assays. Binding of mAb CO17-1A and GA733 to purified tumor antigen was determined in ELISA as described previously (28). Wells of microtiter plates were coated with various concentrations (0.2–5 μg/ml) of antigen or BSA. Binding of mAb or normal mouse IgG (0.4–10 μg/ml) was detected by alkaline phosphatase-conjugated goat anti-mouse F(ab')₂ antibody (Cappel Labs, Durham, NC) and substrate. Absorbances were measured at 405 nm in a TiterTek ELISA reader (ICN Biomedicals, Inc., Costa Mesa, CA).

Binding of mouse sera to GA733-2 antigen-positive SW1116 cells and antigen-negative melanoma cells WM9 (29) was determined in mixed hemadsorption assay as described previously (30). Adherent tumor cells were incubated with various dilutions of mouse sera obtained before and after immunization with native GA733-2 antigen, recombinant GA733-2E antigen, or BSA. Binding of serum antibodies to the cells was detected by sheep RBC that had been bound with mouse anti-sheep RBC antibodies and rabbit anti-mouse IgG antibodies serving as indicator cells. Statistical significance of immunological data was calculated with Student's t test.

RESULTS

PCR Synthesis of pGA733-2 Extracellular Domain. Oligonucleotide primers were designed for PCR (31) synthesis of a truncated cDNA that encodes the amino-terminal signal peptide sequence and the extracellular 242-amino acids of the antigen (Fig. 1). In the 5' end oligonucleotide sequence (primer 1), a PstI restriction site was placed 11 base pairs upstream of the inherent initiation codon. The positioning of primer 1 considerably reduces the GC-rich 5' untranslated sequences, which is thought to impair the translational level of the recombinant product (22). At the 3' end, a synthetic oligonucleotide (primer 2) introduced a termination codon prior to the start codon of infection. The positioning of primer 2 placed an XbaI restriction site immediately adjacent to the stop codon. The expected ~800-base pair cDNA band was visualized upon ethidium bromide-stained agarose gel electrophoresis (Fig. 1).

Isolation of pGA733-2F and pGA733-2E Baculovirus Recombinants. The pGA733-2F full-length cDNA was inserted into the XbaI site of the baculovirus transfer vector pVL1392, downstream of the AcNPV polyhedrin promoter (Fig. 2). The restriction enzymes PstI and XbaI were used to subclone the GA733-2E PCR product into pVL1392 (Fig. 2). Although T. aquaticus polymerase is known to produce misincorporations (32), DNA sequence analysis of the pVL1392-2E recombinant containing...
the PCR-derived insert indicated 100% identity to the native GA733-2 cDNA sequence (10).

Since homologous recombination between the recombinant plasmid transfer vectors and the baculovirus genome is a very rare event, 2 rounds of enrichment for recombinant virus by dot-blot hybridization preceded 1–2 rounds of visual screening for occlusion body-negative recombinant viral plaques (not shown). The GA733-2E baculovirus recombinant was purified in the same manner.

Expression of Recombinant GA733-2F and GA733-2E Antigens. Sf9 insect cells infected with GA733-2F and GA733-2E purified recombinant virus were found by immunoblot analysis to be capable of expressing recombinant antigens carrying the epitope defined by mAb GA733 (Fig. 3). Additional immunoblot experiments established that the recombinant antigens have a second epitope defined by mAb M77 and M79 (3); reactivity with mAb 323/A3 (6) was also observed (not shown).

Detergent extracts of cells infected with GA733-2F baculovirus (Fig. 3, Lane 4) exhibited antigen that appeared heterogeneous in size (34, 37, 40, 44, and 76 kDa), with the major M40 kDa species comigrating with the native glycoprotein expressed by SW948 colorectal carcinoma cells (Fig. 3, Lane 6). Sf9 control cells infected with wild-type AcNPV produced no protein bound by mAb GA733 (Fig. 3, Lane 5). The high-molecular-mass species (76 kDa) seen in GA733-2F-infected cells (Fig. 3, Lane 4) may represent an antigen dimer. The high-molecular-mass species has been observed previously in purified native preparations (1) and thus does not result from modification by insect cells. The antigen expression level of GA733-2F (Fig. 3, Lanes 2 and 4) was not significantly affected by its 5’ GC-rich untranslated sequence (see vector design in “Materials and Methods”) in comparison to GA733-2E (Fig. 3, Lanes 1 and 3), which lacks these sequences. In comparison to cell lysates, the supernatants of GA733-2F-infected cells (Fig. 3, Lane 2) showed recombinant 40 kDa protein in low amounts. This probably results from cell shearing and cell death during the course of infection, liberating protein from the membranes to the supernatant medium. Thus, as expected, GA733-2F encoding the full-length transmembrane antigen was confined to the cell. Although an immunolocalization of antigen at the cell surface was not performed, mammalian proteins generally segregate in insect cells as in mammalian cells (33).

As was seen with the GA733-2F antigen, multiple species of the GA733-2E antigen are also apparent. The detergent extract of cells infected with GA733-2E baculovirus has minor (29, 35 kDa) and a major (M31 kDa) monomeric species and possible dimer (62 kDa) and tetramer (112 kDa) species (Fig. 3, Lane 3). As anticipated, the GA733-2E baculovirus-infected cells exhibited a greater amount of antigen in the culture supernatant (Fig. 3, Lane 1), compared to GA733-2F (Fig. 3, Lane 2). The GA733-2E antigen found in the supernatant appears less heterogeneous, with the 31-kDa species predominating (Fig. 3, Lane 1). Thus, by removal of sequences for the transmembrane and cytoplasmic domains, GA733-2 was converted from a type I membrane protein into a secreted protein.

N-Glycosylation of Recombinant Antibodies. Antigen heterogeneity was studied by culturing infected Sf9 cells in the presence of two different concentrations of tunicamycin (Fig. 4). Immunoblot analysis with mAb GA733 demonstrated a more uniform appearance of both recombinant antigens. N-Linked glycosylation appeared to have been blocked almost entirely at concentrations of 2 μg/ml of tunicamycin.

The diversity of subspecies around the major 40-kDa antigen species of GA733-2F (Fig. 4, Lane 8) was reduced to a predominating 34-kDa species (Fig. 4, Lanes 6 and 7). This is in agreement with the calculated protein molecular mass of 34.9 kDa.
Fig. 3. Immunoblot of SF9 cells infected with recombinant viruses. Protein aliquots (50 μg) were electrophoresed on a nonreducing SDS-polyacrylamide gel, electroblotted, and reacted sequentially with mAb GA733 and alkaline phosphatase-conjugated anti-mouse IgG. E, GA733-2E; F, GA733-2F; S, culture supernatant; L, infected SF9 cells lysed with NP40; WT, wild type AcNPV infected; SW948, native GA733-2 antigen from SW948 colorectal carcinoma cells; all molecular masses in kDa.

Fig. 4. N-Glycosylation inhibition of recombinant antigens. Infected SF9 cells were subjected to N-glycosylation inhibition. Protein aliquots (40 μg) were immunoblotted, reacted with mAb GA733, and then alkaline phosphatase-conjugated anti-mouse IgG. TM, tunicamycin; in Lanes 1, 3, and 6, TM = 2 μg/ml; in Lanes 2, 4, and 7, TM = 5 μg/ml. S, culture supernatant; L, infected SF9 cells lysed with NP40; SW948, native antigen from SW948 colorectal carcinoma cells; all molecular masses in kDa.

for the antigen (10). Faint additional bands can possibly be attributed to the minimal residual N-glycosylation permitted by the concentrations of tunicamycin administered to the SF9 cells. The 76 kDa putative antigen dimer (Fig. 4, Lane 8) was found to be reduced to 63 kDa by tunicamycin (Fig. 4, Lanes 6 and 7).

The major 31 kDa form of GA733-2E (Fig. 4, Lane 5) was reduced to a 29-kDa species (Fig. 4, Lanes 1–4), which is consistent with a protein molecular mass of 29.8 kDa calculated from the truncated cDNA sequence. The putative 62 kDa antigen dimer (Fig. 4, Lane 5) was reduced to 55 kDa (Fig. 4, Lanes 1–4). Hence, the diversity of subspecies of both recombinant antigens found in the immunoblot analysis are the result of different stages of the glycosylation process of the insect cell and do not represent different protein primary structures.

Purification of Recombinant Antigen. Secretory GA733-2E was purified and used for further immunological studies. The full-length recombinant GA733-2F antigen was not further analyzed. mAb affinity chromatography of supernatants from large-scale suspension cultures infected with GA733-2E baculovirus yielded an average of ~1 mg of secreted antigen per liter. An aliquot of affinity-purified GA733-2E was electrophoresed on a SDS-polyacrylamide gel and stained with silver nitrate (Fig. 5). A 31-kDa monomer, the 62 kDa putative dimer, and minor species characteristic of the various stages of glycosylation of GA733-2E were observed. Thus, the affinity-purified GA733-2E antigen preparation was found to be free of contaminating proteins. Treatment of GA733-2E protein with or without mercaptoethanol prior to electrophoresis had no effect on the 62-kDa species (Fig. 5, Lanes 1 and 2, respectively). This implicates dimerization mechanisms other than disulfide bond formation.

Immunoreactivities of Native and Secreted Recombinant Antigens. Maximal binding reactivities of mAb CO17-1A and GA733 to native GA733-2 antigen, recombinant GA733-2E
antigen, or BSA (negative control) are shown in Table 1. Both mAbs specifically bound to native and recombinant antigen preparations, as indicated by the absence of binding of either mAb to BSA or of normal mouse IgG to tumor antigen. mAb binding to either of the two GA733-2 antigen preparations was both mAb and antigen concentration dependent (data not shown). Recombinant GA733-2E antigen showed significantly higher \((P < 0.05)\) binding reactivity than the native GA733-2 antigen; this was consistent with 2 different batches of each antigen. This phenomenon may be related to the different methods used in the preparative isolation of native membrane antigen and secreted recombinant antigen. Both the immunoblot and ELISA data indicated that the processes of antigen truncation and expression in insect cells yielded a recombinant antigen with epitopes recognized by mAb raised against native antigen.

**Immunogenicities of Native and Secreted Recombinant Antigens.** In mice, both the native GA733-2 antigen and the recombinant GA733-2E antigen induced antibodies binding to antigen-positive SW1116 colorectal carcinoma cells (Fig. 6) but not to antigen-negative melanoma cells WM9 (not shown). Such antibodies were not induced by immunizations with BSA (Fig. 6). The serum end point dilutions \([i.e., \text{the highest dilutions of experimental sera showing significant} (P < 0.05) \text{binding versus control sera obtained either before antigen or after BSA immunization}]\) were similar \([~1:204,800]\) for both groups immunized with either native GA733-2 antigen (1 µg/injection) or recombinant GA733-2E antigen (2.5 µg/injection) (Fig. 6). Furthermore, both antigen preparations induced similar \((P > 0.05)\) concentrations of anti-colorectal carcinoma cell antibodies at all serum dilutions tested. Concentrations of colorectal carcinoma cell-binding antibodies induced with native GA733-2 antigen were somewhat dependent on the dose of the administered antigen (1 µg dose < 5 µg dose, \(P < 0.05\), at serum dilutions of 12.8, 51.2, and \(204.8 \times 10^{-3}\)). Hence, as is often the case with proteins expressed in the baculovirus system, the immunogenicities of recombinant and native antigens are similar (20).

**DISCUSSION**

The experimental active immunotherapy of cancer with purified tumor-associated antigens will require that antigen cDNA be molecularly cloned and expressed, since these antigens cannot be produced in sufficient quantities from cultured human tumor cells. In this study, the baculovirus expression system was chosen to produce recombinant GA733-2 carcinoma-associated antigen, because of the capability of baculovirus to direct the abundant expression of immunogenic recombinant protein.

To simplify the purification of a type I membrane protein, we applied PCR technology to obtain a secretable variant of that protein. The positioning of the 3′-oligonucleotide primer for the PCR synthesis of the extracellular domain of the GA733-2 antigen was determined by hydrophobicity analysis, which located a 23-amino acid hydrophobic domain flanked by positively charged residues (10). This region was interpreted as representing the transmembrane domain. The data presented here support our model for the membrane topology of the GA733-2 antigen (10). The applicability of this approach to other type I membrane proteins will be a function of the

**Table 1 Immunoreactivities of native and secreted recombinant antigens**

<table>
<thead>
<tr>
<th>Antibody*</th>
<th>GA733-2</th>
<th>GA733-2E</th>
<th>BSA</th>
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<tr>
<td>CO17-1A</td>
<td>0.60</td>
<td>1.21</td>
<td>0.07</td>
</tr>
<tr>
<td>GA733</td>
<td>0.86</td>
<td>1.30</td>
<td>0.02</td>
</tr>
<tr>
<td>Normal mouse IgG</td>
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<td>0</td>
<td>0</td>
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*All values were corrected for buffer controls; target antigens at 5 µg/ml.
accuracy of computer programs in locating their transmembrane domains.

The advantage of GA733-2E is that detergent-free, recombinant antigen can be purified from serum-free culture media by one-step mAb affinity column chromatography. Thus, antigen can be obtained without the necessity of extensive culturing of human carcinoma cell lines and laborious extraction procedures. Still, the baculovirus expression system could be optimized to yield higher levels of expression of GA733-2E, inasmuch as it was not significantly overexpressed by insect cells relative to mammalian SW948 cells. Analysis of independent baculovirus recombinants and a time course analysis of antigen levels in the supernatant may improve yields. Since the later stages of baculovirus infection may negatively affect the host cell secretory pathway, the application of a nonlytic expression system may be beneficial (34). In this approach, the baculovirus IE1 promoter directs the continuous expression of secretory glycoprotein in stably transformed insect cells.

The GA733-2 recombinant antigens produced by infected Sf9 cells resemble the antigen produced by human cells in several respects. The GA733-2F antigen comigrated with native antigen in immunoblot analysis. Both recombinant antigens underwent N-linked glycosylation. The GA733-2E recombinant antigen was shown to be immuno-reactive to mAb in vitro. As determined in either immunoblotting or ELISA, the recombinant antigens react with a group of 4 mAbs defining 3 different epitopes (GA733, CO17-1A, and M77/M79). The secreted recombinant antigen preparation described here meets an important preclinical criteria for a potential antigen vaccine, namely, the ability to elicit antibodies which can bind to human colorectal carcinoma cells. Both native and recombinant antigen elicited antibodies in mice which specifically and significantly bound to colorectal carcinoma cells at serum dilutions as high as 1:204,800.

Experimental and clinical trials will follow to assess the ability of the recombinant antigen GA733-2E to confer protective immunity. Currently, we are establishing an animal tumor model in which protective effects of immunizations with the GA733-2E antigen can be evaluated. To date, other human tumor-associated antigens, the epithelial tumor antigen expressed by breast carcinomas (35) and the melanoma-associated glycoprotein p97 (36, 37), have been expressed in the vaccinia virus vector system. Recombinant p97 antigen has induced specific humoral, cellular, and protective immunity in mice, and humoral and cellular immunity in monkeys (36), which emphasizes the potential usefulness of recombinant human tumor-associated antigen as vaccines for cancer patients.

Furthermore, for use in biological, immunological, or physical (i.e., crystallography) assays, the preparative isolation of secretable protein domains containing a specific region of interest (i.e., epitopes) implies extended possibilities to study the nature and function of other membrane proteins.

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