Induction of Antitumor Immunity by an Anti-Idiotype Antibody Mimicking Carcinoembryonic Antigen

Shehla Pervin, Maia Chakraborty, Malaya Bhattacharya-Chatterjee, Hasan Zeytin, Kenneth A. Foon, and Sunil K. Chatterjee

Department of Obstetrics and Gynecology [S. P. S. K. C.], Department of Internal Medicine, Division of Hematology and Oncology [M. C., M. B-C., K. A. F.], Department of Microbiology and Immunology [H. Z.], and The Lucille Parker Markey Cancer Center, [S. P., M. C., M. B-C., H. Z., K. A. F., S. K. C.], University of Kentucky Medical Center, Lexington, Kentucky 40536-0096

ABSTRACT

Carcinoembryonic antigen (CEA) is a tumor-associated antigen expressed on most gastrointestinal adenocarcinomas and is a putative target for cancer immunotherapy. We developed a murine monoclonal anti-idiotype (anti-Id) antibody, 3H1, which mimics a specific epitope of CEA, for cancer immunotherapy. In this study, the efficacy of 3H1 as a tumor vaccine was evaluated in a murine tumor model. In this model, the murine colorectal cancer cell line MC-38 was transduced with the human CEA gene and injected into syngeneic C57BL/6 (H-2b) mice. Immunization of naive mice with 3H1 conjugated with keyhole limpet hemocyanin Freund’s adjuvant induced humoral and cellular anti-3H1 as well as anti-CEA immunity. Mice immunized with 3H1 were protected against a challenge with lethal doses of MC-38-cea, whereas no protection was observed when 3H1 vaccinated mice were challenged with CEA negative MC-38 cells or when mice were vaccinated with an unrelated anti-Id antibody and challenged with MC-38-cea cells (P < 0.003). These data demonstrate that the 3H1 vaccine can induce protective CEA-specific antitumor immunity.

INTRODUCTION

Active specific immunotherapy is an attractive approach to cancer therapy in the adjuvant setting. This therapy is intended to boost the host’s antitumor immune response, in contrast to passive immunotherapy, which requires the infusion of large doses of antibodies or cells. Classic active immunotherapy is based on immunization with TAAs extracted from tumors or TAA obtained by recombinant DNA technology. There are several problems with the use of TAA from tumors: (a) it is difficult to obtain sufficient quantities of purified TAAs for immunization; (b) TAAs are often chemically ill defined and not easily reproduced; and (c) use of TAAs as an antigen has the potential for transmitting putative contaminating tumor viruses. Although this difficulty can be circumvented by the use of TAAs prepared by recombinant technology, there are insufficient data from trials with recombinant TAAs to fully assess the potential of this approach.

One area of active immunotherapy involves the use of anti-idiotype antibodies. This idea is based on Jerne’s network concept (1), which can be summarized as follows. A given antibody (Ab1) reacts with epitopic determinants on an antigen. Although antigenic determinants are mostly found on foreign macromolecules, structural determinants on the variable regions of an Ab1 can also serve as determinants that are recognized by a second antibody (Ab2). The “epitopes” on an Ab1 that are recognized by an Ab2 are called idiotopes, and the Ab2 is an anti-idiotype antibody. The Ab2 can be classified into three types based on the region of the variable domain of the Ab1 they recognize: (1) Ab2α recognizes idiotopes that are outside the antigen binding site. Ab2β recognizes the binding site of Ab1 and resembles the original epitope recognized by Ab1. If the target idiotope is close to the binding site so that it can interfere with antigen binding, it is called Ab2γ (2). Because Ab2β represents essentially the internal image of the antigen, this idiotopic antibody can be used as a surrogate TAA. This cascade of complementary idiotopes is the basis of making idiotype vaccines for cancer as well as for bacterial, viral, or parasitic infections (reviewed in Ref. 3). Our goal is to use this immunomodulatory approach for cancer patients.

There are several advantages for the use of a surrogate TAA such as an Ab2β for cancer immunotherapy. Ab2β is more explicitly “foreign” than is TAA. The TAAs are often part of “self” and usually evoke a very poor immune response in a tumor-bearing host due to a state of T cell-mediated suppression (4, 5) as well as to the development of antigen tolerance (6). An internal image antigen expressed in a different molecular environment may overcome immunosuppression in the host by stimulating “silent clones” and/or by allowing T-cell help to become active, making the overall immune response stronger (7). Furthermore, the immunological status of a cancer patient is often suppressed and only able to respond to certain T-dependent antigens and not to other antigen forms.

We have generated a murine monoclonal Ab2β designated 3H1 (8), which mimics a distinct epitope of the TAA, CEA. CEA is present at high density on tumor cells of more than 95% of colorectal, 70% of lung adenocarcinoma, and 50% of breast cancer patients (9–11). We have shown previously that 3H1 can induce anti-CEA antibody in small animals (8) and nonhuman primates (12). In a Phase I clinical trial of advanced colorectal cancer patients, we further demonstrated that 3H1 can induce anti-CEA immunity in humans (13). In these studies, although induction of anti-CEA immunity by the surrogate antigen could be demonstrated, a definitive antitumor effect of the induced immunity could not be shown. This was in part due to the fact that suitable animal models were not available for the preclinical studies, and the cancer patients included for the Phase I study were all, of necessity, advanced stage patients with large tumor burdens and limited life expectancy. In this report, we have used a murine model to demonstrate that immunity induced by multiple injections of the Ab2β, 3H1, can protect mice against a challenge by lethal doses of CEA-positive tumor cells.

MATERIALS AND METHODS

Materials. Purified CEA was obtained commercially from Rougier Bio- tech (Montreal, Quebec, Canada). This material was isolated from a human liver metastasis of colon adenocarcinoma by perchloric acid extraction and was purified twice by ion-exchange chromatography followed by gel filtration and high-performance liquid chromatography. Purity of this preparation was checked by high-performance liquid chromatography, SDS-PAGE, and immunoprecipitation (8). The monoclonal anti-CEA antibody, 8019 (IgG1κ) has been described previously (8). MC-38 murine colon adenocarcinoma (14) and human CEA transduced murine carcinoma cells, MC-38-cea (15), were kindly...
provided by Dr. Jeffrey Schlom (National Cancer Institute, NIH). Female C57BL/6 mice (6–8 weeks of age) were obtained from Harlan Bioproducts for Science, Inc. (Indianapolis, IN). Cell culture media were from Life Technologies, Inc. [H]Thymidine, [3H]KI and [51-Cr]sodium chromate were obtained from DuPont NEN.

**Vaccine.** Procedures for the purification of anti-idiotypic antibody 3H1 have been described (8). Two control anti-idiotypic antibodies were also used in this study. Of these, 11D1O (16), mimicks an epitope related to a breast cancer-associated antigen, human milk fat globule, whereas 1A7 (17) mimics a melanoma-associated antigen, ganglioside GQ2. Anti-idiotypic antibodies were conjugated to KLH by glutaraldehyde, and the conjugates were dialyzed extensively against PBS (18).

**Immunization.** The first immunization with an idiotypic conjugate was performed i.p. after emulsification with Freund’s complete adjuvant. The dose of immunogen per mouse was adjusted to contain 50 μg of immunoglobulin protein per 100 μl of PBS. Subsequent immunizations were performed s.c., using the same dose, after emulsification with Freund’s incomplete adjuvant. Sera were drawn from the tail vein before each immunization and were stored at −20°C.

**Radioimmunoassay for Detection of Anti-3H1 Antibodies (Ab3).** Falcon plates (96-well) were coated with 250 ng/well of the anti-idiotypic antibody overnight at 4°C. After blocking with 1% BSA in PBS for 2 h at room temperature, the plates were washed extensively with PBS. Sera were pooled from three mice and diluted with PBS (40–320-fold), and 50 μl/well were added. After incubation at room temperature for 2 h, the plates were washed with PBS. Fifty μl of [3H]-labeled antibody (100,000 cpm/well) were added to each well, and the incubation was continued at room temperature for another 2 h. After washing with PBS, counts remaining in the wells were measured in a gamma counter. Assays were performed at least in triplicate, and samples with a SD less than 10% were used to calculate the mean.

**ELISA for Detection of Anti-CEA Antibodies.** ELISA plates (96-well) were coated with 100 μl of a solution of purified CEA, 250 ng/well overnight at 4°C. Wells were blocked with 1% BSA in PBS for 1 h at room temperature. Fifty μl of pooled serum after dilution with PBS (20–160-fold) were mixed with 50 μl of 0.1% Tween-20%/BSA in PBS and added to each well. After incubation at room temperature for 2 h, the plates were washed with 0.05% Tween 20%/1% BSA in PBS. One hundred μl of goat anti-mouse IgG conjugated with alkaline phosphatase (1:1,000-fold dilution) was added to each well. The plates were incubated at room temperature for 2 h. After a thorough washing, 100 μl of phosphatase substrate (Sigma Chemical Co.) dissolved in diethanolamine buffer (50 mg of substrate per 50 ml of buffer) were added to each well. The absorbance at 415 nm was determined in an ELISA reader after 30 min at room temperature. Assays were performed at least in triplicate for each sample, and values within a 10% variance were included for calculation of the mean.

**Idiotype Analysis of Ab3.** ELISA plates (96-well) were coated with 100 μl of a solution of 8019 (Ab1) or 3H1 in PBS and blocked with BSA as described above. To each well, 50 μl of diluted serum and 50 μl of [125I]-labeled antibodies (3H1 or 8019, as appropriate) containing 100,000 cpm in 1% BSA in PBS were added simultaneously. After incubation at room temperature for 2 h, the plates were washed, and the radioactivity was determined as described above. For determining the inhibition of binding of [125I]-labeled 8019 to CEA, CEA-positive MC-38-cea cells (1 × 105/well) were placed in cell culture plates (no. 107 assay plates with filter; V.P. Scientific, Burlingame, CA), reacted with 50 μl of diluted mouse serum plus 50 μl of [125I]-labeled 8019 solution in 1% BSA-PBS, at room temperature for 2 h. The filter from each well was taken out for determining the radioactivity after thorough washing. Pooled serum from normal mice was used as control in these experiments.

**Immune Flow Cytometry.** Both CEA-negative MC-38 and CEA-positive MC-38-cea cells (1 × 106 cells/tube) were reacted with 100 μl of either undiluted mouse serum or 100 μl of PBS or 100 μl (2 μg) of Ab1 (8019) for 1 h at 4°C. After washing, the cells were incubated with goat antiserum F(ab')2 IgG-FITC-labeled antibody (Tago, Inc., Burlingame, CA) for 30 min at 4°C. A filter from each well was taken out for determination of total 51Cr release, 100 μl of 1% Triton X-100 were added to each well. Sera from mice immunized with PBS were used as control. All assays were performed at least in triplicate, and specific lysis was calculated by:

\[
\%\text{ release} = \frac{\text{Experimental release} - \text{spontaneous release}}{\text{Total release} - \text{spontaneous release}} \times 100
\]

**Murine Model for Determining the Efficacy of 3H1 Vaccine.** A murine tumor model expressing human CEA was developed by Dr. Jeffrey Schlom at the NIH. Murine colorectal carcinoma cells, MC-38, were transduced with human CEA (15). The transduced cell line, MC-38-cea, constitutively express CEA in culture. Dr. Schlom kindly provided us with the CEA-transduced and nontransduced cell lines. When these cells are injected s.c. (5 × 106 cells/mouse) into syngeneic C57BL6 (H-2b) mice, tumors develop in 100% of the mice within 10–15 days.

**Cell Culture.** MC-38 and MC-38-cea were grown in DMEM containing 10% FCS, 1% L-glutamine, 100 μg/ml penicillin, and 0.25 μg/ml streptomycin. MC-38-cea were cultured in the presence of 200 μg/ml neomycin analogue G-418. Cells (50–60% confluent) were harvested from flasks following a brief treatment with trypsin.

**Survival of Mice Immunized with 3H1 and Control Vaccine.** Female C57BL/6 mice (6–8 weeks of age) were immunized with 3H1-KLH conjugate or control vaccines (11D10-KLH) as described above. A sham vaccination with PBS was also performed for comparison. Each group of immunized mice was further divided into two equal groups for tumor challenge. Tumor challenge was performed with 5 × 106 CEA-positive MC38-cea or CEA-negative MC-38 cells. Growth of tumor and survival were monitored daily, and tumor volume was determined weekly.

**Statistical Evaluation.** Statistical evaluation was performed using SigmaStat software (Jandel, San Rafael, CA). P < 0.05 was considered to indicate statistical significance.

**RESULTS**

**Development of Anti-Anti-Idiotype Antibody.** To induce Ab3 production in the sera, we immunized C57BL/6 mice with 3H1-KLH conjugate. Ab3 produced in the sera of these allogeneic mice was estimated by aRIA using serum pooled from three randomly selected mice, as described in “Materials and Methods.” Data in Fig. 1A show that Ab3 is detectable in the sera of immunized mice even after a 300-fold dilution. 3H1 induced low but detectable levels of Ab3 in 18 of 20 mice after two weekly immunizations. However, significant Ab3 was generated after immunization three times, and the level peaked after 5 weeks of immunization (Fig. 1B). Two controls were used in this assay. In the first control (Ab2-C1), plates were coated with anti-idiotypic 1A7, which mimicks an epitope on the ganglioside Gb2 (17), and the binding of labeled 3H1 was determined after incubation with 3H1-immunized mice sera. For the second control, plates were coated with 3H1, but sera from mice immunized with 1A7 (five immunizations) were used for the 3H1 binding assay. Radioac-
ANTITUMOR IMMUNITY BY ANTI-IDIOYPE ANTIBODY

sera from mice after five weekly immunizations with the isotype matched anti-idiotype 1A7 conjugate were used as the control in this assay. ELISA with this control serum after a 1:20 dilution had an absorbance of 0.18 at 405 nm, which was less than the value obtained using the preimmune serum (Fig. 2B).

Idiotype Analysis of Ab3. Idiotype of Ab3 induced in mice by 3Hl vaccine was analyzed by the inhibition of binding Ab1 to Ab2 by the mouse serum as described in “Materials and Methods.” Results of these experiments are summarized in Table 1. Binding of Ab1(8019) to 3H1 was inhibited by the serum from five of five immunized mice, regardless of which of the antibody was used to coat the plates (Table 1), suggesting that Ab3 may recognize the same epitope as Ab1 and may thus contain Ab1’ antibodies. Inhibition of binding of 125I-labeled 8019 to CEA was determined by using CEA-positive MC-38-cea cell. Once again, sera from five of five mice inhibited this binding, and the inhibition was significant, even after 80-fold dilution of the serum. These results suggested that the Ab3 induced in mice by the 3H1-KLH conjugate share the same idiotype as Ab1.

Development of Ab1’. Ab3 antibody induced by Ab2β is polyclonal, and a subgroup of the Ab3 has a similar binding site (paratope) as Ab1. Because of this paratope similarity, this type of Ab3 is also called the Ab1’ to indicate that it might differ in its other idiotopes from Ab1. Ab1’ recognizes the cognate TAA and is the only antibody in the Ab3 population that is likely to have the antitumor property. Ab1’ was assayed by ELISA as described in “Materials and Methods.” Low and variable levels (A405 nm of 0.21 ± 0.01; dilution, 1:20) of Ab1’ appeared in 6 of 20 mice after two weekly immunizations. Significant levels of Ab1’, however, appeared after the third immunization, and the levels peaked (A405 nm of 0.56 ± 0.06; dilution, 1:20) around the 6th or 7th week in virtually all animals immunized with the 3H1 vaccine (Fig. 2). Ab1’ can be detected in the sera of immunized mice, even after a 80–160-fold dilution (Fig. 2A).
Table 1  Idiotypic analysis of Ab3 induced in mice immunized with 3H1-KLH conjugate

<table>
<thead>
<tr>
<th>Serum dilution</th>
<th>8019 coat/3H1 binding</th>
<th>3H1 coat/8019 binding</th>
<th>Inhibition of binding to CEA-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>87 ± 4</td>
<td>89 ± 3</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>40</td>
<td>78 ± 8</td>
<td>85 ± 4</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>80</td>
<td>64 ± 9</td>
<td>72 ± 7</td>
<td>14 ± 4</td>
</tr>
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* Procedures for the analysis of Ab3 induced in mice immunized with 3H1 vaccine are described in "Materials and Methods." Serum from each of the five mice was separately assayed to obtain the mean and S.E. In the Ab1-Ab2 binding inhibition assays, inhibition by control normal serum pool varied between 10 and 15%, and inhibition was complete with 2.5 μg of 8019. In the cell binding inhibition assay, inhibition by normal serum was observed with background level.

Fluorescence Intensity

Fig. 3. Immunofluorescence analysis of CEA-positive MC-38-cea and CEA-negative MC-38 cells after reaction with mice sera. The assay was described in "Materials and Methods." A—C, incubation of MC-38-cea cells: A, sera from mice before and after six immunizations with 3H1-KLH conjugate; B, PBS and monoclonal anti-CEA antibody 8019; C, sera from mice before and after six immunizations with isotype-matched, unrelated anti-idiotype antibody, 1A7-KLH. D—F, incubation of MC-38 cells: D, before and after six immunizations with 3H1-KLH conjugate; E, PBS and 8019; F, before and after six immunizations with 1A7-KLH.

Flow Cytometric Analysis of Ab1'. To further verify the nature of Ab1' induced by 3H1 vaccination, flow cytometric analysis of preimmune sera and pooled sera from three mice after six immunizations with the 3H1-KLH conjugate was performed. Results presented in Fig. 3 demonstrate that sera from mice immunized with the 3H1 vaccine can bind to the MC-38-cea cell surface (Fig. 3A) identical to the monoclonal anti-CEA antibody, 8019 (Fig. 3B), whereas no binding was observed with preimmune sera or sera of mice immunized with isotype-matched anti-idiotype antibody, 1A7 (Fig. 3C). CEA-negative MC38 cells did not bind sera from mice immunized with 3H1 vaccine (Fig. 3D), monoclonal anti-CEA antibody 8019 (Fig. 3E), or with sera of mice immunized with 1A7 (Fig. 3F).

Development of Cellular Immunity. To evaluate the induction of cellular immunity, spleen cells from sham-vaccinated mice and those from mice immunized with the 3H1 vaccine were prepared. Spleens from at least three mice selected at random were pooled for use in this assay. T-cell proliferation was carried out as described in "Materials and Methods" using CEA and 3H1 as stimulants. For positive controls, 2 μg of phytohemagglutinin were used in these experiments. Mean counts from at least triplicate wells, with SD within 10%, were used for the determination of stimulation indices. Results in Fig. 4 show that significant proliferation takes place using 3H1 as a growth stimulant following week 3 of immunization (>4 times control), but a maximum index is obtained from spleen cells isolated from mice immunized for 6 weeks. Stimulation indices with CEA follow a similar pattern, and after 6 immunizations, the index was more than six times the control. Although the degree of stimulation of spleen cells by CEA varied, the results were qualitatively similar in several independent experiments. Spleen cells from PBS-vaccinated mice showed negligible stimulation with either 3H1 or CEA. The stimulation index of spleen cells from mice after six immunizations with 3H1-conjugate, in the presence of 1A7 as the stimulant in the assay, was 4.2 ± 1.1, which is significantly (P < 0.001) lower than the value obtained with 3H1 as the stimulant (13.2 ± 1.4).

ADCC of the Sera from Immunized Mice. To determine whether the Ab1' generated by 3H1 immunization is cytolytic for CEA-positive tumor cells, such as MC-38-cea, ADCC was determined as described in "Materials and Methods." Results of the ADCC experiments are summarized in Fig. 5. Although the extent of specific cell lysis by sera from each individual mouse varied (33 ± 7, 1:5 dilution; 13.2 ± 5, 1:10 dilution), significant ADCC was observed in five of five mice. Negligible ADCC was observed with sera from mice immunized with PBS (Fig. 5).

Protection of 3H1 Immunized Mice against Tumor Challenge. Experiments described above show that after five to six weekly immunizations, anti-3H1 humoral and cellular responses reached their maximum levels. Anti-CEA immune responses follow a similar pattern. If the immunity induced is effective and specific for CEA, the
Fig. 5. ADCC by serum from immunized mice. Means (bars, SD) of specific lysis by each vaccine group consisting of five mice are shown. Sera were obtained from mice immunized six times with either the 3H1 vaccine or PBS. Specific cell lysis was determined as described in "Materials and Methods" using MC-38-cea as target cells. Sera were diluted 1:5 and 1:10 with PBS.

CEA-positive tumor should be rejected if implanted after this period. Growth of CEA-negative tumors should remain unaffected by this immunization. Mice immunized by six weekly immunizations with 3H1 vaccine were challenged with lethal doses of CEA-positive MC-38-cea cells or control CEA-negative MC-38 cells. In Fig. 6, results of the survival experiments are shown in a Kaplan-Meier plot. In the experiment described in Fig. 6A, 24 mice were immunized with 3H1 immunogen, as described in "Materials and Methods," and divided into two groups of 12 mice each. One group was challenged with MC-38-cea, whereas the second group was challenged with MC-38 cells. MC-38 tumors grew in the latter mice without any sign of regression, and all of the mice died within 22 days. However, in the group of mice challenged with MC-38-cea, the tumor grew for about 10 days after the challenge and became hemorrhagic with signs of tumor rejection; then tumor disappeared within 3 days. In the absence of further treatment with 3H1, however, tumors regrew at the challenge site. Although all of the mice challenged with MC-38 died within 22 days after challenge, 11 of 12 mice challenged with MC-38-cea survived more than 50 days (P < 0.001).

A group of 10 mice was immunized with the isotype-matched control anti-idiotypic antibody, 11D10. In this vaccine group, five mice were challenged with MC-38 cells, and five were challenged with MC-38-cea cells. The results are shown in Fig. 6B. Although mice challenged with MC-38-cea survived slightly longer than those challenged with MC38 (one remained alive for 50 days), the difference in survival between these two groups was not statistically significant (P > 0.3).

Another group of 11 mice were mock vaccinated with PBS. In this group, six mice were challenged with MC-38-cea cells, and five were injected with MC-38. The survival of these mice is shown in Fig. 6C. Except for one mouse challenged with MC-38-cea, which survived more than 50 days, all remaining mice died within 23 days (P > 0.3).

To determine the efficacy of 3H1 vaccine against established tumors, we performed some preliminary experiments. Mice were injected with $5 \times 10^6$ MC-38-cea cells, and anti-idiotypic antibody therapy was started 3 days after the tumors were injected. Mice were treated by injection with either the 3H1-KLH conjugate or the 11D10-KLH conjugate every 4 days at the tumor injection site for six courses of treatment. Initially, tumors developed in both groups at the same rate. On completion of the six courses of treatment, tumors of six of

Fig. 6. Survival of immunized mice after challenge with tumor cells. Mice were immunized by six weekly injections of: A, 3H1-KLH; B, isotype-matched anti-idiotypic antibody 11D10 conjugated to KLH; and C, PBS as described in "Materials and Methods." One week after the final immunization, mice were injected s.c. with $5 \times 10^6$ MC-38 or MC-38-cea cells. Tumor development and survival were monitored daily.
nine mice treated with the 3H1 vaccine became necrotic and showed signs of regression. In the control group, only one of eight mice showed regression. Although a small number of animals was used in these preliminary experiments, the data are interesting and worth repeating. Experiments are under way with a larger number of animals, using different doses of vaccine administered by different routes.

DISCUSSION

The results described in this communication demonstrate that the anti-idiotypic antibody 3H1, mimicking human CEA, can induce humoral and cellular immune responses in C57BL/6 mice, which inhibit the development of tumors after challenge with lethal doses of syngeneic tumor cells. These therapeutic effects are antigen specific, because tumor development was not prevented when the immunized mice were challenged with the same tumor cells that were CEA negative. A therapeutic effect was not observed in mice vaccinated with an isotype-matched unrelated anti-idiotypic antibody. Low levels of Ab3 were detected in 18 of 20 mice after two to three immunizations and reached their maximum level following five to six immunizations. Ab1' levels paralleled Ab3. T-cell proliferation with CEA as the stimulant also peaked after six immunizations to a maximum of six times the control. Thus, to obtain maximum cellular and humoral immune responses, five to six weekly immunizations were necessary.

For CEA to stimulate splenocytes from 3H1-vaccinated mice, it is necessary that 3H1 have amino acid sequence homology to CEA. Comparison of the amino acid sequence of 3H1 complementarity determining regions with the amino acid sequence of CEA identified several regions of homology (19). Among these, a peptide present in the light chain complementarity-determining region 2 showed very high homology to CEA. It is of interest that, when this peptide was used as a stimulant in the T-cell proliferation assay of peripheral blood mononuclear cells from colorectal cancer patients undergoing 3H1 therapy, a significant proliferation was observed in peripheral blood mononuclear cells from patients after 3H1 therapy compared to before therapy (19).

Ab3 induced in mice by 3H1-KLH vaccine inhibited the Ab1-Ab2 binding in this system (Table 1). Binding of Ab1 to CEA-positive MC-38-cea cells was also significantly inhibited (Table 1). These results suggested the induction of Ab1'-like antibodies in the immunized mice. To further confirm whether the induced Ab1' can bind to CEA expressed on the tumor cell surface, we analyzed the sera from immunized mice by immune flow cytometric analysis using both MC-38 and MC-38-cea cells. These results confirmed that the relevant Ab1' had been induced by 3H1 immunization. We have shown previously that in small animals and nonhuman primates, the 3H1 vaccine can induce Ab1' (8, 12). The induction of cellular immunity by 3H1 in mice was not explored previously. In advanced colorectal cancer patients, alum-precipitated 3H1 induced Ab1' in 9 of 12 patients and CEA-specific T-cell proliferation in 4 patients (13). Although 3H1 induced human antımouse antibody in these patients, Ab1' induction was unaffected in these patients. This Phase I trial, however, was designed to determine the safety of the vaccine and examine the induction of immunity in advanced colorectal cancer patients. There was no toxicity in 3H1-immunized patients, although CEA was a "self" antigen for them. The antitumor efficacy was not tested in this advanced group of patients but is now being tested in patients in an adjuvant setting with minimum tumor burden. Although these T-cell results in mice may not be directly extrapolated to humans, these results are encouraging and suggest that immunization with an Ab2β has the potential for cancer therapy, particularly in the minimum tumor burden adjuvant setting.

For several reasons, we consider CEA an excellent target for active immunotherapy with anti-idiotypic antibody. CEA is one of the most well-characterized TAAs. It is a Mr 180,000 glycoprotein, its gene has been cloned and sequenced (20–22). CEA is expressed at high density on the surface of a vast majority of human colorectal carcinomas, gastric and pancreatic tumors, and also on other adenocarcinomas such as breast and lung cancer (9–11). Trace amounts of CEA are present in some normal colonic epithelial cells, whereas it is highly expressed in the fetal gut tissues (23, 24). CEA has been shown to function as a homotypic intercellular adhesion molecule (25). It has been speculated that alterations in its expression in carcinoma cells may lead to a general derangement in cell adhesion and consequent disruption of normal cell-cell interactions, resulting in metastasis. This speculation is supported by results of studies in murine models as well as in cancer patients. For example, the levels of CEA produced by human colorectal cancer cell lines directly correlated with their ability to form hepatic metastasis in nude mice following intrasplenic injection (26, 27). i.v. injection of CEA in nude mice prior to intrasplenic injection of tumor cells has also been shown to enhance metastases from weakly metastatic colorectal cancer cell lines (28).

Results of studies in cancer patients showed that the prognosis of colorectal cancer patients was generally poor following surgery, when their serum CEA levels were high (29). Moreover, tumor cells isolated from patients with high serum CEA levels develop more frequent hepatic metastases in nude mice than those from patients with low serum CEA (30). Recently, it has been demonstrated that human colorectal carcinoma cell lines transfected with cDNA encoding CEA showed significant increase in liver metastases following intrasplenic injection into nude mice, which could be inhibited by anti-CEA antibodies (31). Our model is not metastatic; therefore, we could not demonstrate any effect of 3H1 in cancer metastasis. However, if anti-CEA antibodies can inhibit tumor metastases (31), anti-CEA antibodies induced by 3H1 immunization in colorectal cancer patients (13) are likely to be beneficial to these patients.

Several monoclonal antibodies recognizing TAAs have shown to mediate ADCC (32–35); none of them were against CEA. Recently, one such antibody (R4) was made against CEA (36). Significant ADCC was invoked in five of five mice by immunization with a 3H1-KLH conjugate. ADCC could be an additional important mechanism for tumor protection by 3H1 immunization. We are currently planning to confirm this observation by adoptive transfer of immune serum into naive mice.

Induction of cellular immunity in cancer patients by anti-Id vaccination has been reported by several laboratories (13, 37–41). Using another Ab2β, a significant correlation was reported between Ab2β-dependent T-cell stimulation and tumor regression in colorectal cancer patients (41). Taken together, these data show that an Ab2β can induce effective T-cell responses.

We are at present determining the subtypes of T cells that were induced to proliferate by CEA, the nature of the cytokines released, as well as the roles of various cytotoxic T cells that may be involved in the eradication of CEA-positive tumors in this model.

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