Antitumor efficacy of CD137 ligation is maximized by the use of a CD137 single-chain Fv–expressing whole-cell tumor vaccine compared with CD137-specific monoclonal antibody infusion

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Abstract

Tumor-destructive immune responses can be generated by engaging CD137 (4-1BB) via infusing a monoclonal antibody specific for CD137 or vaccinating with a single-chain Fv (scFv) CD137-expressing whole-cell tumor vaccine. We assessed whether such a vaccine can induce tumor rejection in the neu-transgenic (neu-Tg) mouse breast cancer model and compared the antitumor efficacy of vaccination with the infusion of a CD137-specific antibody. Mammary carcinoma cells (MMC) from a neu-Tg mouse were transfected to stably express surface scFv derived from the anti-CD137 rat hybridoma 1D8 or 3H3. The anti-CD137 scFv-expressing cells were rejected when transplanted into neu-Tg mice by a mechanism that involved both CD4+ and CD8+ T cells, and vaccination with such cells delayed the outgrowth of MMC cells transplanted 3 days previously. T cells from neu-Tg mice that had been vaccinated proliferated and produced IFN-γ when stimulated by MMC but not by antigen-negative variant breast cancer cells that did not express the neu tumor antigen. In addition, antibodies binding to the MMC but not to antigen-negative variant cells were detected in sera from some but not all of the immunized mice. Complete regression of s.c. transplanted MMC tumors was observed in mice repeatedly immunized against MMC-1D8 starting on the day the MMC cells were transplanted. In contrast, repeated administration of either of two different anti-CD137 monoclonal antibodies did not induce complete tumor regression, although tumor growth was delayed. [Mol Cancer Ther 2006;5(1):149–55]

Introduction

Many tumor antigens, including antigens encoded by HER-2/neu, are self-proteins and induce peripheral tolerance that prevents the generation of a tumor-destructive immune response. One approach to circumvent tolerance is to activate tumor-specific T cells via certain costimulatory receptors. Much attention has been given to CD137 (4-1BB), which was first detected on activated T lymphocytes (1, 2) and subsequently on natural killer cells (3), monocytes (4, 5), and dendritic cells (6). Engagement of CD137 can up-regulate T-cell responses, increase natural killer cell reactivity, and depress the formation of T-cell-dependent antibodies (2, 3, 7–9). Administration of anti-CD137 monoclonal antibodies (mAb) and vaccination with tumor cells transfected to express the CD137 ligand or with a combination of cDNAs encoding tumor antigen together with costimulatory signals by CD137 antigen together with costimulatory signals by CD137 ligand and CD80 or CD86 can break tolerance and induce a tumor-destructive immune response in some mouse models (10–16). The antitumor response by injecting anti-CD137 mAb is often greater than that by vaccinating with tumor cells transfected to express CD137 ligand. For example, mice with established sarcoma Ag104 can be treated by administration of anti-CD137 mAb (10) but not by vaccination with tumor cells expressing CD137 ligand (11, 15). To construct a vaccine that engages the immune system similar to an agonistic mAb, tumor cells can be transfected to stably express, at their surface, single-chain Fv fragments (scFv) from the given mAb (17, 18). Such a vaccine may be therapeutically more effective than a systemically given mAb by delivering tumor antigens together with signals that engage CD137 and may avoid the inhibitory effect of the mAb on endogenous antibody formation.

Based on these premises, Ye et al. transfected cells from the M2 clone of the K1735 mouse melanoma to express anti-CD137 scFv and showed therapeutic efficacy against small M2 tumors growing s.c. or in the lung (19). Furthermore, cells from sarcoma Ag104 became immunogenic when transfected to express anti-CD137 scFv3 but not when transfected to express CD137 ligand (11).

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Note: M.L. Disis and I. Hellstrom contributed equally to this work.

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2Yang et al., unpublished findings.

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We have evaluated in the neu-transgenic (neu-Tg) model (20–23) the therapeutic efficacy of vaccinating mice with tumor cells expressing anti-CD137 scFv and have compared vaccination with administration of anti-CD137 mAbs. Although neu-Tg mice are peripherally tolerant to neu-expressing mammary cancers for which neu is a self-antigen (21), tolerance can be broken and neu is a target for tumor rejection (14, 22–24).

Materials and Methods

Mice

Specific pathogen-free breeder FVB/N-TgN (MMTV/neu) 220 mice, which are transgenic for rat neu (20), were purchased from the Jackson Laboratory (Bar Harbor, ME) and bred at the University of Washington Animal Facilities under specific pathogen-free conditions. Females, 8 to 12 weeks old, were used for the present experiments according to approved protocols in accordance with recommendations for the proper use and care of laboratory animals.

Tumor Lines

Mammary carcinoma cells (MMC), a transplantable mouse mammary carcinoma line that overexpresses rat neu, and a transplantable mammary carcinoma line, antigen-negative variant (ANV), which does not overexpress rat neu, were used. MMC was derived from a spontaneous mammary tumor from a FVB/N-TgN 220 mouse, and ANV was obtained by transplanting MMC cells into nontransgenic FVB mice whose immune system selects for cells that lack neu (23, 25). Both MMC and ANV express MHC class I, although their expression of class II is low. AgT04 (26) is a sarcoma that occurred spontaneously in a C3H/HeN (H-2K) mouse. It expresses class I MHC molecule and was used as a genetically mismatched control tumor line in CTL assays. Cultured tumor cells were maintained in RPMI 1640 supplemented with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum. Tumor cells sterilized by incubation with mitomycin C (Sigma, St. Louis, MO; with 20% fetal bovine serum.

Vectors and Transfection of MMC Cells

Genes encoding scFv from rat anti-mouse CD137 hybridomas 1D8 (2, 10) and 3H3 (2) were obtained, and membrane binding PLNCX-1D8scFv (PLNCX-1D8) and PLNCX-3H3scFv (PLNCX-3H3) vectors were constructed as published (17–19). A PLNCX-control vector (PLNCX-control) was obtained, which, like the other constructs, expresses hIgG Fc tail, hCD80 transmembrane, and a cytoplasmic domain but lacks anti-CD137 scFv. It was made by deleting the scFv from the PLNCX-1D8 construct after restriction endonuclease digestion with AccI and AclI according to the sequence of PLNCX-1D8. Vectors were transfected into RetroPACK PT67 packaging cells (BD Clontech, Mountain View, CA) by LipofectAMINE 2000 (Invitrogen, Carlsbad, CA). MMC cells were transfected, selected, and tested as in previous studies (19) and are called MMC-1D8, MMC-3H3, or MMC-control. All three lines express similar levels of human Fc.

Production and Purification of Anti-CD137 mAb

Hybridoma 1D8 (5) is no longer available, and our experiments were done with mAbs obtained from either of two other rat hybridomas that make agonistic anti-mouse CD137 mAbs 2A (rat IgG2a; ref. 13) and 3H3 (rat IgG2a; ref. 2). The mAbs were obtained and purified as described previously (2, 10, 13), and their binding to CD137 was verified by flow cytometry with COS7-mCD137 cells that had been transfected to express mouse CD137.

Flow Cytometry

Fluorescence-activated cell sorting analyses were done with an EPICS CL machine (Coulter, Denver, CO). For detection of the neu protein, Ab4 (Oncogene Science, Cambridge, MA) was used as primary antibody and phycoerythrin-conjugated goat anti-mouse IgG (Biosource International, Camarillo, CA) as secondary antibody. To detect antibodies in sera, MMC and ANV cells were stained by mouse serum as the primary antibody and with a fluorescein-conjugated goat anti-mouse IgG (Biosource International) as the secondary antibody.

Protection against Tumor Outgrowth In vivo

Mice were immunized by s.c. transplantation on one side of the back with 2 × 106 mitomycin C–treated (or live; see Results) MMC-1D8, MMC3H3, or mitomycin C–treated MMC-control cells. Unless otherwise stated, they were immunized thrice at 7- to 10-day intervals and 10 days later transplanted s.c. on the contralateral side of the back with MMC (2 × 106 per mouse) or ANV (1 × 106 per mouse). Tumor size was assessed by measuring twice weekly the two largest perpendicular diameters and reported as average tumor area (mm2) ± SD. For prevention of i.p. growth of tumor cells injected i.p., MMC-1D8 immunized mice were injected i.p. with 1 × 106 MMC cells and survival was determined.

To compare the therapeutic activity of anti-CD137 mAb and vaccination with MMC-1D8 cells, one group of mice was injected s.c. with 2 × 106 MMC cells and subsequently transplanted s.c. on the contralateral side of the back with mitomycin C–treated MMC-1D8 or MMC cells; this was repeated at 3- to 4-day intervals. Another group that had been transplanted s.c. with the same dose of MMC cells was injected i.p. with mAb 3H3 or 2A, starting on the day of tumor transplantation. Control mice received rat IgG. Mice were followed for tumor growth and survival, and mice whose tumors had regressed were observed for a minimum of 90 days and evaluated for toxicity.

In vivo Depletion of CD4+ and/or CD8+ T Lymphocytes

T cells were depleted as described (27) by injecting mice i.p. thrice with mAb to CD4 (GK1.5, rat IgG2b; Dr. R.S. Mittler, Emory University, Atlanta, GA) or CD8 (169-4, rat IgG2a; American Type Culture Collection, Manassas, VA) or with a mixture of the two at 0.5 mg per mouse for 3 consecutive days. This was followed by 0.5 mg of each mAb every third day. Rat IgG (Sigma and Rockland, Gilbertsville, PA) was used as control. On day 12, spleen cells from similarly injected mice were analyzed by flow...
cytometry to verify that the depletions were efficient. On day 13, mice (n = 5 per group) were transplanted s.c. with MMC-1D8 cells (2 × 10^6 per mouse) and followed for tumor outgrowth.

**In vitro Assays of T Cells**

Proliferation assays were done as described (10). Two to 4 weeks after the last immunization, splenocytes were harvested and seeded into 96-well flat-bottomed plates (2.5 × 10^5 per well) together with 2.5 × 10^4 mitomycin C–treated MMC or ANV cells. After incubation for 72 hours, triplicate cultures were pulsed for 16 to 18 hours with 1 mCi [3H]thymidine (Amersham Pharmacia Biotech, Piscataway, NJ), the uptake was measured, and mean and SD were calculated. ELISPOT kits (Cell Sciences, Norwood, MA) were used to measure the production of IFN-γ by spleen cells from immunized mice. To investigate the specificity of the IFN-γ response, spleen cells (5 × 10^5 per well) from naive, MMC-control, and MMC-1D8 immunized mice were collected 2 weeks after the last immunization and cultured with MMC or ANV cells at a 10:1 ratio for 20 hours in IFN-γ antibody-coated plates, and counting of spots was done using an Image analyzer. CTL activity was determined by a standard 4-hour ^51Cr release assay (28).

**Statistical Analysis**

Statistical significance was determined by applying the Student’s t test. P < 0.05 was considered as significant.

**Results**

**Immunization with MMC-1D8 Cells Induces Rejection of MMC Cells**

We first compared the growth in naive neu-Tg mice of (wild-type) MMC, MMC-control, and MMC-1D8 cells. MMC and MMC-control cells grew progressively in all mice with similar growth kinetics. In contrast, MMC-1D8 cells formed tumors that regressed within 20 days. MMC cells (2 × 10^6 per mouse) transplanted 10 to 14 days after the rejection of MMC-1D8 were consistently rejected (data not shown).

We next explored whether vaccination of neu-Tg mice with mitomycin C–treated MMC-1D8 compared with MMC-control cells caused the rejection of s.c. transplanted MMC cells (2 × 10^6 per mouse). In the experiment shown in Fig. 1A and B, mice vaccinated with MMC-control cells developed tumors, whereas four of five mice that had been immunized against MMC-1D8 rejected the transplanted MMC cells and remained tumor free until the experiment was terminated 90 days later. Mice vaccinated against MMC-1D8 did not reject transplanted ANV cells (which do not express neu), although ANV tumors grew slightly slower in mice immunized against MMC-1D8 than in mice immunized against MMC-control (data not shown).

MMC cells form tumors when transplanted i.p. and thus provide a model for tumors, which like ovarian carcinomas, primarily metastasize to the peritoneal cavity and often overexpress neu.4 Therefore, mice that had been vaccinated twice by s.c. transplantation of live MMC-1D8 cells were injected i.p. with 10^6 MMC cells on the day of their second vaccination and subsequently monitored daily; five naive mice and five mice immunized with mitomycin C–treated MMC-control mice were used as controls. The mice were monitored daily and mice were sacrificed (followed by necropsy) when there was evidence of tumor growth, like swelling of the abdomen, difficulties to eat and drink, or weight loss. As shown in Fig. 1C, all eight mice vaccinated with MMC-1D8 survived when the experiment was terminated 90 days later. Necropsy of these mice revealed no evidence of tumor. In contrast, all five naive mice and all five mice that had been immunized with MMC-control cells had to be terminated within 33 days when they had solid tumors in their abdomen and some also had ascites.

**Immunization against Small Established Tumors**

Figure 2 shows an experiment in which mice were transplanted s.c. with 2 × 10^6 MMC cells and 3 days later immunized by s.c. transplantation of 2 × 10^6 mitomycin C–treated cells from MMC-1D8, MMC-3H3, or MMC-control.

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4 K. Knutson, unpublished findings.
There was an identical and significant growth retardation in the two former groups. Vaccination with either MMC-1D8 or MMC-3H3 cells was ineffective when done 7 days after transplantation of MMC.

Transplanted MMC-1D8 Cells Are Rejected by a Mechanism for Which CD4+ and CD8+ T Cells Are Needed

To deplete CD4+ or CD8+ T cells separately or together before transplantation of 2 × 10^6 MMC-1D8 cells, naive neu-Tg mouse were injected i.p. with rat mAbs to the respective cell populations, whereas control mice were injected with rat IgG. Fluorescence-activated cell sorting analysis of spleen cells from similarly injected mice showed that >95% of the targeted cell populations had been depleted (data not shown). As shown in Fig. 3, removal of both CD4+ and CD8+ T cells prevented regression of transplanted MMC-1D8 cells, which grew even faster than MMC or MMC-control cells that had been transplanted to naive neu-Tg mice. MMC-1D8 cells formed tumors in mice depleted of either CD4+ or CD8+ T cells, but these tumors grew much more slowly than in the group lacking both CD4+ and CD8+ T cells with the slowest growth observed in mice whose CD8+ T cells were intact. The MMC-1D8 cells were rejected within 2 weeks by all mice injected with rat IgG.

Vaccination of neu-Tg Mice with MMC-1D8 Cells Induces neu-Specific T Cells and Antibodies

MMC (neu+) and ANV (neu−) cells were used as stimulators in proliferation and ELISPOT assays with spleen cells from mice immunized against MMC-1D8. There was a 3-fold (P < 0.04) increase in the proliferation of spleen cells from mice immunized against MMC-1D8 and cocultivated with mitomycin C–treated MMC compared with ANV cells (P = 0.04).

CTL responses were detected with spleen cells that had been cocultivated with mitomycin C–treated MMC cells for 5 to 6 days after harvest from mice immunized against MMC-1D8, including mice that have rejected MMC cells (Fig. 4C). The splenocytes displayed a statistically significant (P < 0.01) CTL activity against MMC also at the lowest E:T ratio tested (5:1) but only at the highest ratio (50:1) against ANV cells, and it was only 50% of that observed against MMC. No significant CTL activity was detected against the MHC class I incompatible Ag104 cells.

Vaccination of neu-Tg mice against MMC-1D8 were tested by flow cytometry for binding to MMC and ANV cells. Figure 5A shows that sera diluted 1:50 from one of five mice immunized against MMC-1D8 and from three of five similarly immunized mice that have rejected a challenge of MMC cells 70 days previously bound to MMC cells. The binding was mediated by IgG antibodies and was equal at dilution 1:25 (data not shown); a low binding was observed at a dilution of 1:100, whereas there was no binding at 1:200. Sera binding to MMC did not bind to ANV cells (Fig. 5B). No antibodies were detected in mice immunized against MMC-control cells.

Vaccination with MMC-1D8 Cells Is Therapeutically More Effective Than Administration of Anti-CD137 mAb

Figure 6 depicts an experiment in which mice transplanted s.c. with 2 × 10^6 MMC cells were repeatedly injected either i.p. with the anti-CD137 mAb 3H3 or s.c. with MMC-1D8 cells starting on the day of transplantation with MMC cells. Whereas MMC grew rapidly in mice that received rat IgG or mitomycin C–treated MMC cells, tumor growth was delayed in mice that had been injected with mAb 3H3 and complete regressions were obtained in four of five mice that had been immunized against MMC-1D8 cells, and these four mice remained tumor free for an against MMC-control. The frequency of positive cells was higher after the spleen cells had been cocultivated with mitomycin C–treated MMC compared with ANV cells (P = 0.04).

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Sera collected 21 to 30 days after three immunizations of neu-Tg mice against MMC-1D8 were tested by flow cytometry for binding to MMC and ANV cells. Figure 5A shows that sera diluted 1:50 from one of five mice immunized against MMC-1D8 and from three of five similarly immunized mice that have rejected a challenge of MMC cells 70 days previously bound to MMC cells. The binding was mediated by IgG antibodies and was equal at dilution 1:25 (data not shown); a low binding was observed at a dilution of 1:100, whereas there was no binding at 1:200. Sera binding to MMC did not bind to ANV cells (Fig. 5B). No antibodies were detected in mice immunized against MMC-control cells.

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observation period of >6 months without detectable side effects. Tumor growth was identical in control mice immunized with mitomycin C–treated MMC cells and in mice that had received rat IgG. It is noteworthy that there was an 10-day lag period before a retarded tumor growth was observed in mice receiving mAb 3H3 and that two additional mAb injections around day 35 when the tumors had become large had no therapeutic effect. Similar results were obtained with anti-CD137 mAb 2A (data not shown). The experiment was repeated twice with similar results.

In another experiment, 10 mice were injected 10 times, at 3-day intervals, with 100 to 300 μg mAb 3H3 at each time. In this case, the mAb-treated mice survived up to 30 days longer than the controls with a similar delay in tumor growth in all the treated mice. However, no complete regressions were observed. Unexpectedly, 4 of these 10 mice displayed significant weakness of their hind legs and had to be euthanized. Necropsy and examination of organs, including brain and nerves, did not reveal the cause of this problem.

No therapeutic effects of administering anti-CD137 mAb were observed against MMC tumors from cells transplanted 3 days previously (data not shown), whereas, as shown in Fig. 2, inhibited tumor growth was seen after vaccination with MMC-1D8 cells.

**Discussion**

We have extended previous findings in the K1735 mouse melanoma model (19) to mammary carcinomas in neu-Tg mice, a biologically relevant model for human breast carcinoma, by showing that immunization with live or mitomycin C–treated MMC-1D8 or MMC-3H3 cells, which express anti-CD137 scFv, causes rejection of transplanted MMC (wild-type) cells, which like many human breast carcinomas overexpress neu. CD4+ and CD8+ T cells were needed for rejection of the MMC-1D8 cells, and spleen cells from neu-Tg mice that had been transplanted with MMC-1D8 proliferated, secreted IFN-γ in response to antigens expressed on MMC cells, and generated specific CTL. Our data imply that tolerance to neu (and perhaps to other tumor antigens as well) in the transgenic mice was broken. However, the effects seen on already established tumors

Figure 4. MMC-1D8 immunized neu-Tg mice mount a T-cell response to MMC cells. **A,** proliferation of spleen cells from mice immunized against MMC-1D8 after *in vitro* stimulation by MMC or ANV cells. The experiment was repeated with similar results. **B,** ELISPOT assays of IFN-γ secretion by spleen cells from mice immunized twice by s.c. transplantation of MMC-1D8 or MMC-control cells or from naive mice. Mice were killed 2 wks after the last immunization and their splenocytes were cultured with mitomycin C–treated MMC or ANV cells (10:1) for 20 h; control wells with medium alone were included. The experiment was repeated with similar results. **C,** CTL activity of spleen cells from mice that had been immunized twice by transplantation of MMC-1D8 cells. Two weeks after the last immunization, the mice were killed and their spleen cells were restimulated *in vitro* for 6 d with mitomycin C–treated MMC cells. Cytolytic activity was measured by triplicate determinations against MMC, ANV, and Ag104 cells at different E:T ratios.

Figure 5. Antibodies binding to MMC cells in a fraction of neu-Tg mice after immunization against MMC-1D8. **A,** neu-Tg mice were immunized twice at 7- to 10-d intervals with MMC-1D8 cells and with control mice being immunized against MMC-control cells. Subsequently, a 1:50 dilution of serum was applied as the primary antibody for flow cytometry with MMC (neu+) and ANV (neu−) cells and using a secondary antibody against mouse IgG. Sera were also tested from five mice from the experiment in Fig. 1A. These sera were harvested 70 d after the first vaccine. The same results were obtained in a repeat experiment. **B,** binding of serum, diluted 1:50, from mice immunized against MMC-1D8 (solid line) to MMC but not to ANV cells. Sera from naive mice (dashed line) were tested as controls.
were modest, and further studies are needed to investigate whether, for example, procedures counteracting the effect of regulatory T cells (29) would improve the therapeutic efficacy of vaccination.

The demonstration that depletion of either CD8+ or CD4+ T cells affects the rejection of MMC1D8 cells by neu-Tg mice is different from observations with the K1735 melanoma where depletion of CD4+ T cells prevented rejection of cells transfected to express anti-CD137 scFv, whereas depletion of CD8+ T cells was ineffective (19). Most likely, the very low MHC class I expression by K1735 prevents it from being an in vivo target for CD8+ CTL, so that tumor rejection is exclusively dependent on CD4+ cells, whereas both CD4+ and CD8+ T cells have been shown to be needed to protect neu-Tg mice from outgrowth of carcinoma cells expressing neu (23, 30).

Antibodies to MMC cells were detected in some of the mice immunized against MMC-1D8 but not in mice immunized against MMC-control cells. Their binding to MM2 cells transfected with the 3H3 and 1D8 vectors were compared with 3H3 and 2A because results obtained with CD137, focusing the immune response on antigens that also tumors growing i.p. can be destroyed by infections. Alternatively, 10 repeated injections of the mAb may have broken tolerance to some normal tissue antigens, analogous to the breakage of tolerance to tumor antigens by engaging CD137. The relatively modest antitumor effects of the mAbs and the observed toxicity is in contrast to observations made in several other systems (10, 15).

The greater antitumor response by vaccinating with tumor cells expressing anti-CD137 scFv over injecting anti-CD137 mAbs may be due to the fact that tumor antigens are delivered together with a signal engaging CD137, focusing the immune response on antigens expressed by the tumor cells, an approach that is also likely to cause less side effects. It is unlikely that the better efficacy of the vaccination via the 1D8 vector is due to differences between the antigen-binding parts of mAb 1D8 compared with 3H3 and 2A because results obtained with MMC cells transfected with the 3H3 and 1D8 vectors were similar. All three mAbs have shown similar antitumor activity when tested in other systems (8, 10). Furthermore, mAb 1D8 was therapeutically inferior to vaccination with 1D8-transfected melanoma cells (19), and mouse melanoma cells transfected to express either 1D8 or 3H3 are equally effective as vaccines.5

We hypothesize that vaccination with autologous tumor cells transfected to express anti-CD137 scFv may prevent or delay relapses in high-risk human patients if they, like the successfully treated mice, have a very small tumor load (e.g., in patients with advanced ovarian carcinoma after they have become ‘clinically tumor free’ following surgery and chemotherapy). Our finding that also tumors growing i.p. can be destroyed by vaccination is encouraging in this context. Because the antitumor effects were observed by engaging CD137 via scFv rather than its ligand, we postulate that recombinant vaccines [e.g., such that comprise cDNAs (14)] will be more effective by choosing the scFv approach over the ligand.

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5 Unpublished data.


Molecular Cancer Therapeutics

Antitumor efficacy of CD137 ligation is maximized by the use of a CD137 single-chain Fv–expressing whole-cell tumor vaccine compared with CD137-specific monoclonal antibody infusion

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