Cancer Immunol Immunother (2000) 49:123–132 © Springer-Verlag 2000

POINT AND COUNTERPOINT

**Haruhiko Maruyama · Jan Zaloudik · Weiping Li
Melinda Sperlagh · Takashi Koido · Rajasekharan
Somasundaram · Stacey Scheck · Marie Prewett
Dorothee Herlyn**

Cancer vaccines: single-epitope anti-idiotype vaccine versus multiple-epitope antigen vaccine

Received: 27 December 1999 / Accepted: 27 January 2000

Abstract In this study, we compared the immunogenicity and tumor-protective activity of anti-idiotypic antibodies mimicking a single tumor-associated epitope and tumor-associated antigen expressing multiple potentially immunogenic epitopes. We focused our study on the colorectal-carcinoma(CRC)-associated antigen GA733 (also known as CO17-1A/KS1-4/KSA/EpCAM). Monoclonal anti-idiotypic antibody (Ab2) BR3E4 was produced against murine anti-CRC mAb CO17-1A (Ab1) in rats. Full-length native GA733 protein was isolated from human tumor cells, and the extracellular domain protein (GA733-2E) was isolated from supernatants of recombinant baculovirus-infected insect cells by immunoaffinity chromatography. The immunomodulatory activity of the Ab2 was compared with that of the antigen, both in rabbits and in mice. Mice, like humans but not rabbits, express a GA733 antigen homologue on some of their normal tissues. Thus, these in vivo models allow the comparison of the immunogenicity of Ab2 and antigen in the presence (mice) and absence (rabbits) of normal tissue expression and immunological tolerance of the GA733 antigen homologue. In rabbits, aluminum-hydroxide(alum)-precipitated native GA733 antigen was superior to alum-precipitated Ab2 in inducing specific humoral immunity. In mice, alum-precipitated recombinant GA733-2E antigen, but not alum-precipitated Ab2, induced specific humoral immunity. However, when the Ab2 was administered to mice in Freund's complete adjuvant, specific humoral immune responses were elicited. Ab2 in complete Freund's adjuvant and GA733-2E in alum were compared for their capacity to induce antigen-specific cellular immunity in mice. Whereas lymphoproliferative responses were obtained with the recombinant antigen only, delayed-type hypersensitivity responses were obtained with both recombinant antigen and Ab2, although these responses were lower than after antigen immunization. The recombinant antigen in alum did not protect mice against challenge with antigen-positive syngeneic murine CRC cells. Similar studies with Ab2 BR3E4 mimicking the CO17-1A epitope were not possible because the tumor cells do not express this epitope after transfection with the human GA733-2 cDNA. However, similar studies with Ab2 mimicking the epitope defined by mAb GA733, which is expressed by the transfected tumor cells, indicated a lack of tumor-protective activity of this Ab2. In contrast, the full-length antigen expressed by recombinant adenovirus inhibited the growth of established tumors in mice. In conclusion, soluble antigen is a more potent modulator of humoral and cellular immune responses than Ab2, both administered in adjuvant. However, for induction of protective immunity, the immunogenicity of the antigen must be further enhanced, e.g., by expression of the antigen in a viral vector.

Key words Anti-idiotypic antibodies · Colorectal carcinoma · CO17-1A/GA733/KSI-4/KSA/EpCAM · Humoral immunity · Cellular immunity

The counterpoint article follows this one

H. Maruyama · J. Zaloudik · W. Li · M. Sperlagh · T. Koido
R. Somasundaram · S. Scheck · M. Prewett · D. Herlyn (✉)
The Wistar Institute of Anatomy and Biology,
36th Street at Spruce, Philadelphia, PA 19104, USA
e-mail: dherlyn@wistar.upenn.edu
Tel.: +1-215-898-3962
Fax: +1-215-898-0980

Introduction

Anti-idiotypic antibodies (Ab2), binding to the antigen-combining site of antitumor antibodies (Ab1), can functionally and structurally mimic tumor antigen. Ab2 have induced antigen-specific humoral, cellular and protective immunity against tumors in animals and in patients (reviewed in [24]). With the availability of tumor antigens, it has become possible to compare the immunogenicity and protective activity of single-epitope Ab2 and multiple-epitope antigen vaccines. The functional differences between these two types of vaccine have been

discussed [13]. Thus, Ab2 vaccines have an intrinsically higher specificity, but lower immunogenicity, than antigen vaccines. These differences probably reflect the targeting of a single epitope by Ab2 vaccines instead of the multiple epitopes targeted by antigen vaccines. The fine specificities of vaccine-induced immune responses to tumors are also more predictable for Ab2 vaccines than for antigen vaccines. Thus, Ab2 vaccines are less likely to induce immunity to antigens other than the original one, whereas antigen vaccines may induce immunity to a variety of epitopes expressed by different antigens of tumor and normal tissues as well as antigens of other origins.

In the present study, we compared the immunogenicity of Ab2, mimicking an epitope on a tumor antigen, with that of the antigen, in mice and rabbits. We focused on the colorectal-carcinoma(CRC)-associated antigen GA733 (also known as CO17-1A/KS1-4/KSA/EpCAM, EGP) [6, 15, 17, 32, 45, 49]. The GA733 antigen is a suitable target for passive and active specific immunotherapy against CRC. The antigen is expressed by more than 90% of CRC tissues derived from various patients [12]. The antigen is also expressed on a few normal tissues, such as colon, pancreas and lung [12], albeit at lower density than on tumor tissues [34, 43]. A phase II/III randomized control clinical trial with monoclonal antibody (mAb) CO17-1A in CRC patients has demonstrated significantly enhanced survival of the treated patients [39]. In initial studies of active immunotherapy, polyclonal goat Ab2 to Ab1 CO17-1A specifically modulated the humoral immune responses of rabbits and cancer patients. However, antibody titers induced by the polyclonal Ab2 were low [19, 20, 33]. In efforts to improve the efficacy of active immunotherapy against the GA733 antigen, we have developed monoclonal Ab2 against Ab1 CO17-1A [28] and isolated full-length, native GA733 antigen and a recombinant extracellular-domain antigen (GA733-2E) [48]. In a homogeneous population of paratopic (combining-site-specific) monoclonal Ab2, every molecule potentially mimics the antigen. In contrast, only a fraction of the antibodies in polyclonal Ab2 preparations (approx. 26% for polyclonal Ab2 CO17-1A [19]) mimic the antigen. Consequently, the fraction of antigen-specific Ab3 induced by monoclonal paratopic Ab2 should be significantly higher than that induced by polyclonal Ab2. Furthermore, variations in antibody composition between various batches of monoclonal Ab2 should be minimal, whereas such variations can be considerable in polyclonal preparations. The GA733 antigen expresses multiple potentially immunogenic epitopes, whereas Ab2 mimics only one epitope. Therefore, we postulated that the antigen may be superior to Ab2 in inducing antitumor immunity.

Monoclonal Ab2 BR3E4 was produced against murine anti-CRC Ab1 CO17-1A in rats [28]. Full-length, native GA733 protein was isolated from human tumor cells and the extracellular-domain protein (GA733-2E) from supernatants of recombinant baculovirus-infected insect cells by immunoaffinity chromatography [48]. The immunogenicity of Ab2 and the antigen was compared in both mice and rabbits. Mice, but not rabbits, express a GA733 antigen homologue on their normal tissues [51]. Thus, these in vivo models allow the comparison of Ab2 and antigen for their immunogenicity in the presence (mice) and absence (rabbits) of normal tissue expression and immunological tolerance of the GA733 antigen homologue [23]. The mouse model closely mimics the situation in humans, whereas the rabbit model is especially useful in determining whether Ab2 can induce antigen-specific immunity across species barriers (i.e., in a species different from the species of origin of Ab1), which is an important criterion for the internal antigen image nature of Ab2 [21]. We previously reported that full-length, native GA733 antigen and extracellular-domain protein GA733-2E can elicit CRC cell-binding antibodies in mice [48]. Furthermore, Ab2 BR3E4 was shown to induce Ab1-like anti-anti-idiotypic antibodies (Ab3) in rabbits [28]. Here we have extended those studies to include the comparison of the capacity of Ab2 BR3E4 and GA733 antigen (native or recombinant) to induce (i) tumor-cell- and antigen-specific humoral immunity, both in mice and in rabbits and (ii) antigen-specific lymphoproliferative and delayed-type hypersensitivity (DTH) responses in mice.

Using these animal models, we demonstrate here that the CO17-1A antigen is a more potent humoral and cellular immune response modulator than monoclonal Ab2 BR3E4, both in mice and in rabbits, although in rabbits the monoclonal Ab2 is more potent than the previously described polyclonal Ab2 [19]. However, the protein is unable to protect mice against a challenge with antigen-positive, syngeneic CRC cells. Induction of protective immunity requires further enhancement of antigen immunogenicity, e.g., by expression of the antigen in a viral vector [26, 31].

Materials and methods

Monoclonal and polyclonal antitumor antibodies

Murine mAb CO17-1A, GA733 and CA19-9 raised against human CRC cells and mAb ME491 against human melanoma cells have been described [1, 15, 17, 29]. mAb CO17-1A and GA733 bind to overlapping epitopes on the same antigen [41]. Polyclonal rabbit antibodies to the GA733 antigen, used in immunohistoperoxidase staining of tissues, were purified from immune rabbit sera (for immunization of rabbits, see below) by protein-A–Sepharose column chromatography and have been described in detail [51].

Cell lines

SW1116 CRC cells and WM9 melanoma cells [16, 29] were maintained in L-15 medium supplemented with 10% fetal bovine serum (FBS). Murine CT26 CRC cells [5] were transfected with GA733-2 cDNA [50] by standard calcium phosphate precipitation and selected with G418 (Gibco BRL, Grand Island, N.Y., USA). Clone CT26-AlGA710-3H was selected because it stably expressed the GA733 antigen both in vitro and in vivo in a mixed hemadsorption assay (MHA) and immunohistoperoxidase assay respectively [35].

However, the transfectants do not express the epitope defined by mAb CO17-1A (our unpublished data), although they do express the mAb-GA733-defined epitope [35].

Antigens

Native GA733 and ME491 antigens were isolated from human SW1116 CRC or WM9 melanoma cell extracts on immunoaffinity columns coupled with mAb GA733 or mAb ME491 [8, 41, 48]. Native GA733 antigen was used for immunization of rabbits. With the availability of recombinant GA733-2E antigen derived from baculovirus [48], a more abundant source of the antigen with less qualitative batch-to-batch variation became available. Recombinant GA733-2E antigen, produced and purified as described [48], was used for all immunizations of mice. The native and the recombinant antigen were indistinguishable in their immunogenicity, as determined by analysis of their capacity to elicit antibodies specifically binding to CO17-1A-antigen-positive CRC cells in mice [48].

Hybridoma production

Production and selection of Ab2 BR3E4 hybridoma cells, and purification and in vitro characterization of the Ab2 have been described in detail [28]. Briefly, Ab2 BR3E4 specifically and significantly inhibited binding of Ab1 CO17-1A to antigen-positive tumor cells, and GA733 antigen almost completely inhibited binding of Ab2 BR3E4 to Ab1 CO17-1A.

Immunizations of rats, rabbits, and mice

Immunization of rats for generation of Ab2 BR3E4 to Ab1 CO17-1A has been described [28]. For immunization of mice and rabbits with Ab2 BR3E4 or CO17-1A antigen, alum was chosen as the primary adjuvant because this adjuvant has been approved by the Food and Drug Administration and because alum-precipitated polyclonal goat Ab2, mimicking the CO17-1A or GA733 epitope have induced antigen-specific immunity in rabbits and cancer patients [18–22, 33, 46]. For induction of Ab3, two rabbits (New Zealand White, Hare-Marland, Hewitt, N.J., USA) were immunized s.c. with 300 μg alum-precipitated Ab2 on day 0, followed by 100 μg of the Ab2 in alum on days 8, 27, 70, and 103. Two control rabbits were similarly immunized s.c. with normal rat IgG [28]. For induction of antibodies to the native GA733 antigen, two rabbits each were immunized s.c. with 5 μg alum-precipitated native GA733 antigen on days 0, 8, and 27. Two control rabbits were similarly immunized with bovine serum albumin (BSA).

For induction of Ab3 in mice, seven mice per group (BALB/c, Harlan-Sprague Dawley, Indianapolis, Ind., USA) were immunized s.c. with 100 μg Ab2 BR3E4 precipitated with alum (five bi-weekly injections). Since alum-precipitated Ab2 did not induce Ab3 in mice (see Results), separate groups of mice were injected with 100 μg antibodies emulsified in complete Freund's adjuvant (CFA) (day 0) or incomplete Freund's adjuvant (IFA) (days 14 and 28). For induction of antibodies to GA733-2E, mice (5–6/group) were immunized s.c. three times at 2-week intervals with 5 μg/mouse of GA733-2E precipitated with alum [48]. Control mice were similarly immunized with BSA.

For induction of proliferative lymphocyte responses by Ab2 BR3E4, mice (5/group) were injected with 50 μg or 100 μg Ab2/mouse s.c. in CFA (day 0) or IFA (days 12 and 33). Separate groups of mice received 100 μg of Ab2 in CFA once. For induction of DTH responses against Ab2, groups of mice (4–5 per group) were immunized s.c. once with 100 μg Ab2 in CFA. Control mice received normal rat IgG in the same dose and adjuvant as the Ab2-immunized mice. For induction of lymphoproliferative responses by antigen, mice (5/group) were immunized s.c. with 5 μg alum-precipitated GA733-2E once. Control mice were similarly immunized with BSA. For induction of the DTH response to GA733-2E, mice (5/group) were immunized s.c. three times at 2-week intervals with 5 μg GA733-2E precipitated with alum. Control mice were similarly immunized with BSA.

For induction of tumor-protective immunity, mice were immunized with Ab2 BR3E4 or GA733-2E according to the schedule, which induced humoral and cellular immunity. Thus, mice were immunized with 100 μg of Ab2 BR3E4 in CFA/IFA on days 0, 12, and 33, or with 5 μg alum-precipitated GA733-2E on days 0, 14, and 28.

Antibody-binding assays

The avidity of the interaction between Ab1 CO17-1A and Ab2 BR3E4 was determined by radioimmunoassay as described in detail [27, 37]. Briefly, Ab2 or control normal rat IgG at various concentrations (50–5000 ng protein/well) was bound to wells of microtiter plates coated with goat anti-rat $F(ab')_2$ antibody (500 ng/well). Ab2 BR3E4 or normal rat IgG at each concentration was then incubated with ^{125}I-labeled Ab1 CO17-1A or control normal mouse IgG at various concentrations (720 000–1250 cpm/well). The Ab2 BR3E4 concentration binding saturating amounts of ^{125}I-Ab1 CO17-1A with high specificity (less than 10% of the input radioactivity of ^{125}I-labeled control mouse IgG bound to Ab2; less than 10% of the input radioactivity of ^{125}I-labeled Ab1 CO17-1A bound to Ab2 BR3E4 in the presence of a 100-fold excess of unlabeled Ab1 CO17-1A) was determined. The avidity of the interaction between Ab2 BR3E4 and Ab1 CO17-1A was calculated according to the method of Scatchard [42] from the binding values obtained with the predetermined amount of Ab2 BR3E4 and various amounts of ^{125}I-labeled Ab1.

All assays for detection of rabbit and mouse Ab3 and antibodies to the CO17-1A antigen have been described [18, 19, 22]. Briefly, binding of rabbit and mouse serum antibodies to cultured tumor cells was determined in a mixed hemadsorption assay using sheep red blood cells (SRBC) sensitized with rabbit (or mouse) anti-SRBC antibody to which goat anti-[rabbit (mouse) IgG] antibody was bound to detect binding of rabbit (mouse) antibodies to tumor cells. Binding of rabbit serum antibodies to isolated native GA733 antigen was determined in an enzyme-linked immunosorbent assay using antigen as the target and peroxidase-labeled goat anti-(rabbit IgG) antibody as tracer, followed by addition of substrate (H_2O_2) and color indicator ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] to detect binding of the rabbit antibodies to the antigen.

Antibody binding inhibition assays

All assays have been described in detail [18, 19, 22]. Inhibition by rabbit Ab3 (or antibodies to GA733 antigen) of Ab1 CO17-1A binding to CRC cells was determined by radioimmunoassay. ^{125}I-labeled Ab1 CO17-1A (or ^{125}I-labeled anti-CRC control mAb CA19-9) at concentrations showing approximately 50% maximal binding (approx. 5000 cpm ^{125}I-mAb/well) was added to CRC cells pre-incubated with sera from rabbits immunized with rat Ab2 BR3E4, normal rat IgG, GA733 antigen or BSA, and inhibition of binding of ^{125}I-Ab1 CO17-1A or ^{125}I-mAb CA19-9 to the cells by the rabbit sera was determined. Sera from rabbits immunized with the rat antibodies were preincubated with normal rat IgG and assayed.

In vitro lymphocyte proliferation test

This assay has been described in detail [22, 35]. Briefly, lymphocytes from spleens or draining lymph nodes obtained from mice 8–12 days after immunization (10^5cells/well) were stimulated in vitro with Ab2, normal rat IgG, GA733-2E (all at 0.2–50 μg/ml), irradiated (400 Gy from a Cs source) CRC SW1116 cells, or control WM9 melanoma cells (0.04×10^3–1×10^3/well), or concanavalin A (0.5 μg/ml) for 3–6 days. Separate cultures were restimulated with the various preparations after 6 days for 2 additional days. In a few

mice, adherent cells from spleens were pulsed with the various antigen preparations and added to the lymphocytes. Activation of lymphocytes was tested in standard [^{3}H]thymidine incorporation test. Results were expressed as a stimulation index [22, 35].

DTH reactions in mice

Immunized mice were challenged intradermally (i.d.) with 1 μg GA733-2E (right ear) or irrelevant ME491 melanoma antigen (left ear). Ear thickness was measured with a caliper (Poco Test, Mitutoyo, Japan) before and 18, 24, and 48 h after challenge. The increase in thickness was calculated for each ear at various times after challenge relative to the thickness before challenge [22, 35].

Immunohistoperoxidase staining of tumor tissues

Twenty-week CT26-ALGA710-3H tumors obtained from syngeneic BALB/c mice were fixed [10% formalin in phosphate-buffered saline PBS] and embedded in paraffin. Sections were incubated with 5% normal goat serum and 1% BSA in PBS for 1 h at room temperature and stained with biotinylated polyclonal rabbit antibody to the GA733 antigen using the Histostain SP kit (Zymed Labs Inc., San Francisco, Calif., USA). Biotinylated normal rabbit IgG was used as a control.

Tumor challenge in mice

The effect of GA733-2E antigen immunization on antigen-positive syngeneic tumor growth was determined in mice. These studies did not include Ab2-immunized mice because the tumor cells do not express the CO17-1A epitope (see above). Immunized mice (4–6/group) were challenged s.c. with 4×10^7 CT26-ALGA710-3H cells expressing the GA733 antigen or with parental, antigen-negative CT26 cells 2 weeks after the last immunization. Tumors were measured with a caliper twice each week for up to 2 months after the challenge [14].

Statistical analyses

Significance of differences between experimental and control values (triplicates) was determined by Student's *t*-test.

Results

Avidity of monoclonal rat Ab2 BR3E4

The avidity of Ab2 BR3E4 binding to Ab1 CO17-1A was 4.5×10^9 M^{-1}, whereas the avidity of GA733 antigen binding to mAb CO17-1A was 0.7×10^8 M^{-1} [38].

Binding of rabbit antibodies elicited by Ab2 BR3E4 or native GA733 antigen to CRC cells

Sera from rabbits immunized five times with alum-precipitated Ab2 BR3E4 or three times with alum-precipitated native GA733 antigen bound specifically (as compared to control sera) to CRC SW1116 cells (Fig. 1), but not to GA733-antigen-negative WM9 melanoma cells (not shown). Sera obtained from the same rabbits before immunization and sera from control immunized rabbits did not bind significantly to CRC cells (less than 5% of cells bound; Fig. 1). Serum titers of anti-CRC antibodies elicited in the two rabbits by Ab2 BR3E4 were approximately 1:15 and 1:45 (Fig. 1A) respectively, whereas the titers of both rabbits immunized with the native GA733 antigen were approximately 1:150 (Fig. 1B). Furthermore, the maximum percentage

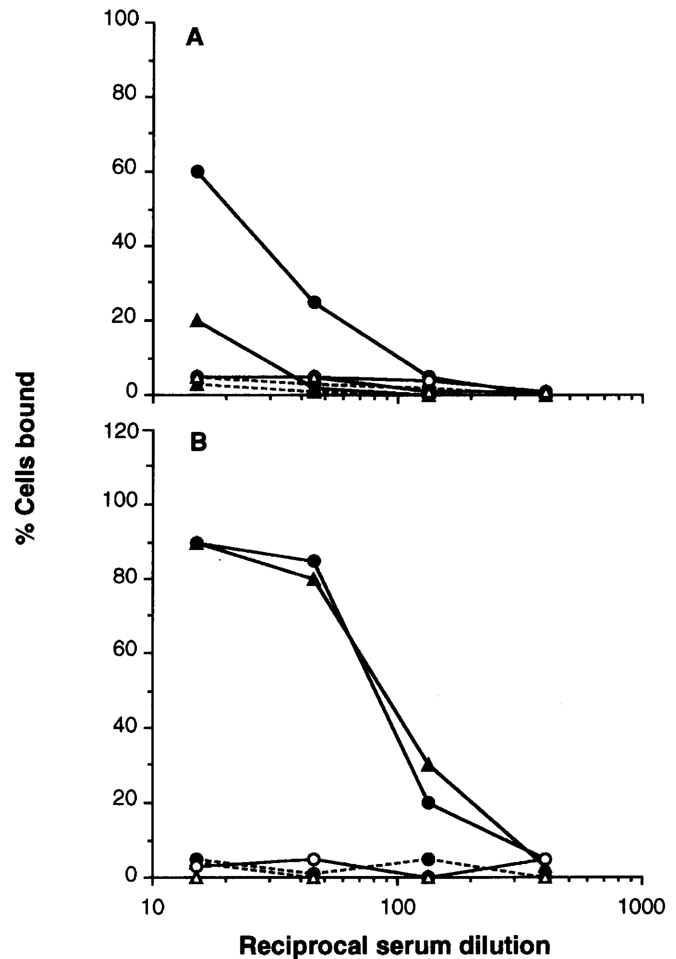

Fig. 1A, B Binding of rabbit sera elicited by Ab2 BR3E4 (**A**) or native GA733 antigen (**B**) to colorectal carcinoma (CRC) cells. Rabbits were immunized s.c. with five injections (1 × 100 μg, 4 × 300 μg) of alum-precipitated Ab2 BR3E4 (●, rabbit 988; ▲, rabbit 989) or normal rat IgG (△, rabbit 3021) on days 0, 8, 27, 70, and 103 (**A**), or three injections of 5 μg alum-precipitated GA733 antigen (●, rabbit 111; ▲, rabbit 112) or bovineserumalbumin (○, rabbit 117; △, rabbit 118) on days 0, 8, and 27 (**B**). Sera obtained from the rabbits before immunization (- - -) or 7–17 days after the last immunization (—) were tested for binding to SW1116 CRC cells in a mixed hemadsorption assay (MHA) using sheep red blood cells (SRBC) sensitized with rabbit anti-SRBC antibody to which goat anti-(rabbit IgG) antibody was bound to detect binding of the rabbit sera to tumor cells. Sera from Ab2- or antigen-immunized rabbits did not bind significantly to GA733-antigen-negative WM9 melanoma cells (not shown). The reciprocal serum endpoint dilution, i.e., the highest reciprocal dilution at which experimental values were more than threefold greater than control values (pre-immune value of corresponding experimental animal or post-immune value of control animals), was approximately 15 and 45 for the two Ab2-immunized rabbits and approximately 150 for the two antigen-immunized rabbits. (SE of triplicate determinations was below 5%)

of CRC cells that bound the rabbit antibodies was 60% for anti-Ab2 sera and 90% for anti-antigen sera (Fig. 1). Both the anti-Ab2 and the anti-antigen sera significantly ($P < 0.05$–< 0.001 versus control sera at 1:25 dilution) inhibited binding of Ab1 CO17-1A (Fig. 2), but not unrelated mAb CA19-9 (not shown), to CRC SW1116 cells. Therefore, the elicited antibodies may bind to the same epitope as Ab1 CO17-1A. The maximal inhibition obtained with the two anti-Ab2 sera was 60% and 38% respectively. Ab3 concentrations and/or avidities may be too low to inhibit binding of Ab1 to tumor cells completely. Sera from both rabbits (rabbits 111 and 112) immunized with the native GA733 antigen inhibited this reaction significantly less than did the serum of one of the two rabbits (no. 989) immunized with Ab2 BR3E4 ($P < 0.05$ and < 0.01; Fig. 2), but not as compared to serum from the other Ab2-immunized rabbit (no. 988). The low inhibitory capacity of the anti-antigen sera most likely reflects the low percentage and/or low avidity of CO17-1A-epitope-specific antibodies among all antibodies induced by the antigen.

Binding of rabbit antibodies elicited by Ab2 BR3E4 or native GA733 antigen to isolated GA733 antigen

Antibodies elicited by alum-precipitated Ab2 BR3E4 (five injections) in rabbits significantly ($P < 0.001$ as compared to control sera) bound to native GA733 antigen (Fig. 3A). However, alum-precipitated native CO17-1A antigen (three injections) was more potent

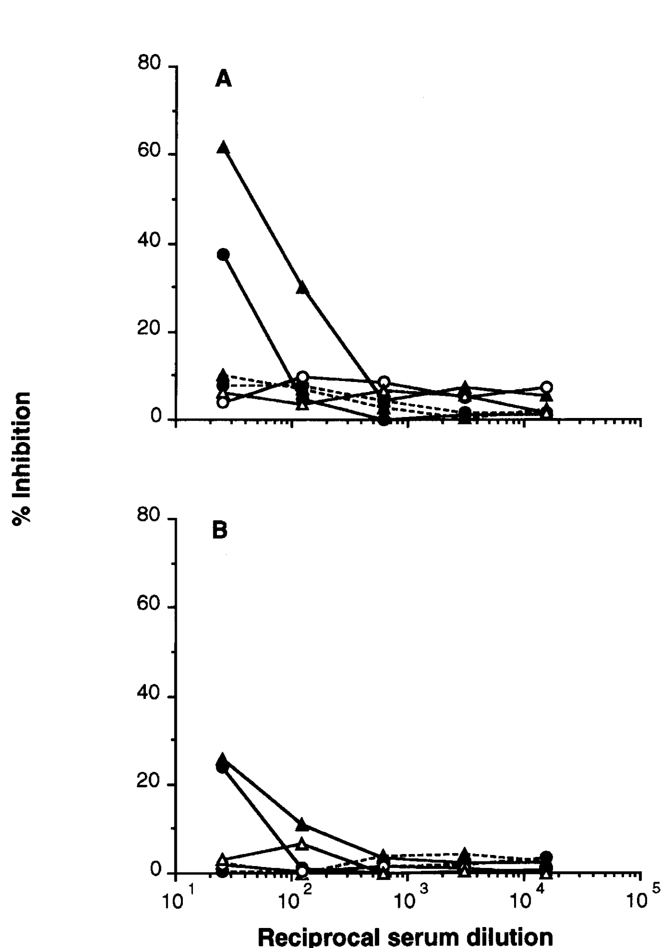

Fig. 2A, B Inhibition of binding of mAb CO17-1A to CRC cells by rabbit sera elicited by Ab2 BR3E4 (**A**) or native GA733 antigen (**B**). Immunization and serum collection were as described in the legend to Fig. 1. Inhibition of binding of ^{125}I-labeled Ab1 CO17-1A to SW1116 CRC cells by the sera was tested in a radioimmunoassay. Sera of Ab2-immunized rabbits were preincubated with 400 μg/ml normal rat IgG before the assay. Binding of ^{125}I-labeled irrelevant mAb CA19-19 to the cells was not significantly inhibited by the immune sera (not shown). Experimental values are significantly ($P < 0.05$–< 0.001; Student's *t*-test) different from the corresponding control values at the lowest serum dilution of 1:25. (SE of triplicate determinations was below 5%)

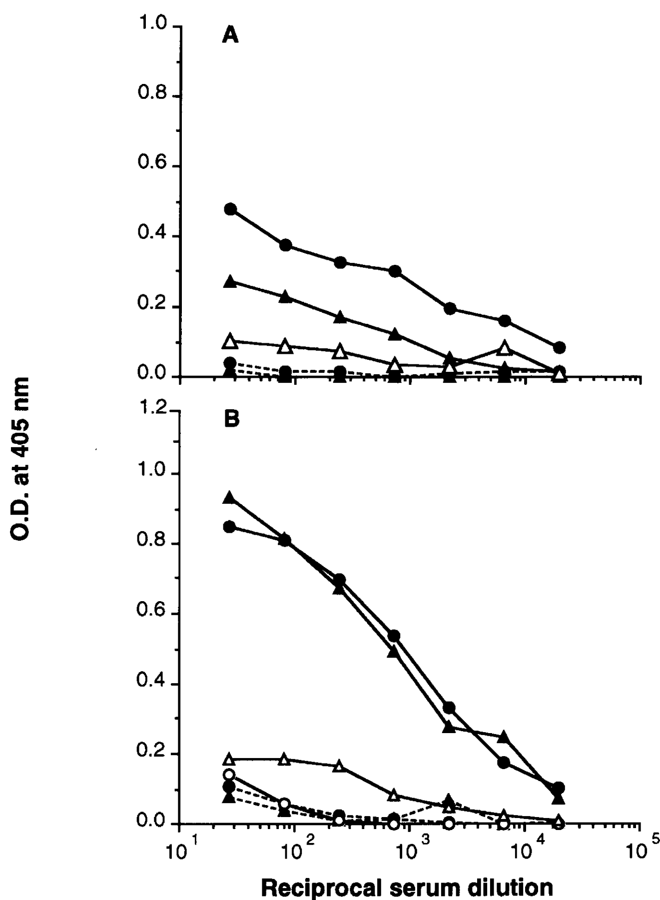

Fig. 3A, B Binding of rabbit sera elicited by Ab2 BR3E4 (**A**) or native GA733 antigen (**B**) to native GA733 antigen. Immunization and serum collection were as described in the legend to Fig. 1. Binding of sera to GA733 antigen was tested in an enzyme-linked immunosorbent assay using the antigen as target and peroxidase-labeled goat anti-(rabbit IgG) antibody as tracer to detect binding of the rabbit antibodies to the antigen. The post-immunization sera did not significantly bind to gelatin (not shown). The reciprocal serum endpoint dilution, i.e., the highest reciprocal dilution at which experimental values differed significantly ($P < 0.001$) from the corresponding control values, was approximately 80 and 800 for the two Ab2-immunized rabbits, respectively, and approximately 7000 for both antigen-immunized rabbits. (SE of triplicate determinations was below 5%)

than Ab2 BR3E4 in its capacity to elicit antigen-specific antibodies in rabbits (Fig. 3B; $P < 0.01$ for comparisons of values of 1:25 diluted post-immunization anti-antigen serum 111 with anti-Ab2 serum 988 or 989, and anti-antigen serum 112 compared with anti-Ab2 serum 988 or 989).

The studies above demonstrate that both Ab2 BR3E4 and the native GA733 antigen can elicit antigen and epitope-specific antibodies, although the antigen seems to be a more potent immunogen in rabbits. The demonstration of immunogenicity of the Ab2 in rabbits, i.e., in a species different from that used for induction of Ab1, suggests that the Ab2 bears the internal image of the CO17-1A epitope [21]. However, rabbits, unlike humans, do not express the CO17-1A antigen on their normal tissues, as determined by immunohistoperoxidase staining of rabbit tissues with polyclonal antibodies to the GA733 antigen [51]. Therefore, rabbits are of limited value as a direct preclinical model of CO17-1A vaccines. A CO17-1A antigen homologue has been described in mice [2, 3, 51], which, therefore provide a relevant in vivo model to compare the immunogenicities of Ab2 and antigen in the immunotolerant host. Mice also are the preferred model to test for induction of cellular immune responses, such as DTH reactions, by Ab2 or antigen. Rabbit skin is hypersensitive to any proteins we have used thus far in i.d. injections, even in the absence of specific immunization (our unpublished results). Furthermore, mice, but not rabbits, allow testing of protective immune responses induced by the vaccines because transplantable tumors are available in mice only. Our studies performed with Ab2 BR3E4 and recombinant GA733-2E in mice are described in the following sections. Recombinant GA733-2E [48], and not native GA733 antigen, was used in all studies in mice because the recombinant antigen is easier to produce in large quantities with minimal qualitative batch-to-batch variations. The two forms of the antigen were indistinguishable in their immunogenicity, as demonstrated by induction of specific humoral immunity to antigen-positive tumor cells by the two preparations in mice [48].

Binding of murine antibodies elicited by Ab2 BR3E4 to CRC cells

Five injections of Ab2 BR3E4 in alum did not induce anti-CRC antibodies in mice (results not shown). However, mice immunized three times with Ab2 BR3E4 emulsified in CFA/IFA developed significant ($P < 0.05$ versus control mice immunized with normal rat IgG) concentrations of antibodies binding specifically to CO17-1A antigen-positive SW1116 CRC cells (Fig. 4) but not to WM9 control cells (not shown). The antibody titers were 1:45 (Fig. 4). These titers were significantly ($P < 0.05$) lower than those previously induced in mice with three injections of GA733 antigen (native GA733 or recombinant GA733-2E) precipitated with alum (titer = 1:100 000 serum dilution [48]).

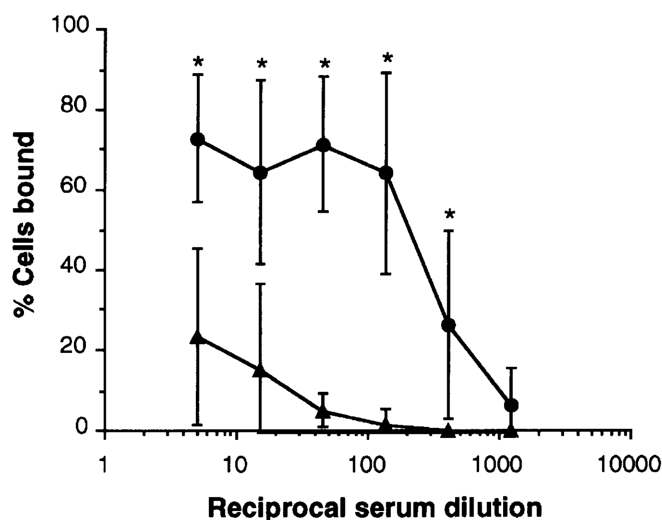

Fig. 4 Binding of mouse sera elicited by Ab2 BR3E4 to CRC cells. BALB/c mice (five per group) were immunized s.c. three times at 2-week intervals with 100 μg Ab2 BR3E4 (●) or normal rat IgG (▲), both emulsified in complete Freund's adjuvant (first injection) or incomplete Freund's adjuvant (subsequent injections). Sera were obtained 10 days after the last immunization and tested for binding to CRC cells SW1116 in MHA using SRBC sensitized with mouse anti-SRBC antibody to which goat anti-(mouse IgG) antibody was bound to detect binding of mouse antibodies to tumor cells. Post-immunization sera from Ab2-immune mice did not bind to WM9 melanoma cells (not shown). Means ± SD of five mice per group. ★ Values significantly ($P < 0.01$) different from the corresponding control values

Cellular immune responses elicited by Ab2 BR3E4 or recombinant GA733-2E antigen in mice

Since Ab2 in CFA/IFA, but not alum-precipitated Ab2, induced antigen-specific humoral immune responses in mice, cellular immune responses to the Ab2 in mice were investigated using CFA and IFA as adjuvants. Ab2 BR3E4, given once or three times in CFA or CFA/IFA respectively, did not induce antigen-specific proliferative lymphocyte (derived from spleen or draining lymph nodes) responses in mice under the various in vitro assay conditions and using the various stimulants described in Materials and methods (not shown). However, GA733-2E precipitated with alum primed murine splenocytes for significant lymphoproliferative responses to in vitro stimulation with the antigen (Fig. 5). Proliferative lymphocyte responses of recombinant GA733-2E-antigen-immunized mice were significantly ($P < 0.01$) higher than the responses of BSA-immunized mice (Fig. 5).

Mice immunized once with Ab2 BR3E4 in CFA developed specific DTH reactions to i.d. challenge with GA733-2E as compared to challenge with ME491 antigen ($P < 0.05$; Fig. 6A). DTH reactions of Ab2-immunized mice to challenge with GA733-2E were also significantly ($P < 0.05$) higher than those mice immunized with of normal rat IgG challenged with the antigen (Fig. 6A). Alum-precipitated GA733-2E also elicited significant and specific DTH reactions in mice, but these reactions were less than those elicited by Ab2 (Fig. 6B).

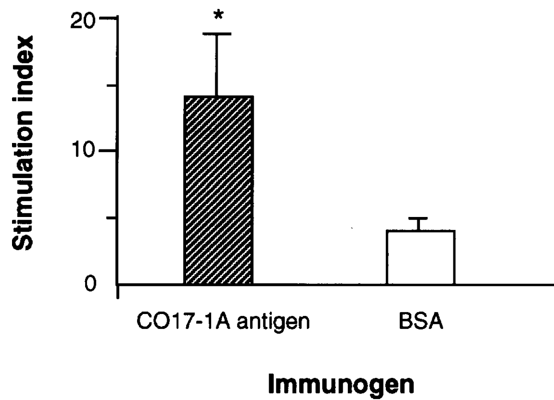

Fig. 5 Lymphocyte-proliferative responses of mice immunized with recombinant GA733-2E. Mice (five per group) were immunized once s.c. with 5 μg alum-precipitated GA733-2E or bovine serum albumin (*BSA*). Ten days later, spleens were removed and single-cell suspensions were stimulated with 5 μg/ml GA733-2E for 3 days, followed by determination of [^{3}H]thymidine incorporation by the cells. Mean ± SD of five mice per group. ★ Value that differs significantly ($P < 0.01$) from the corresponding control value. This value also differs significantly ($P < 0.05$) from the culture medium control value (not shown)

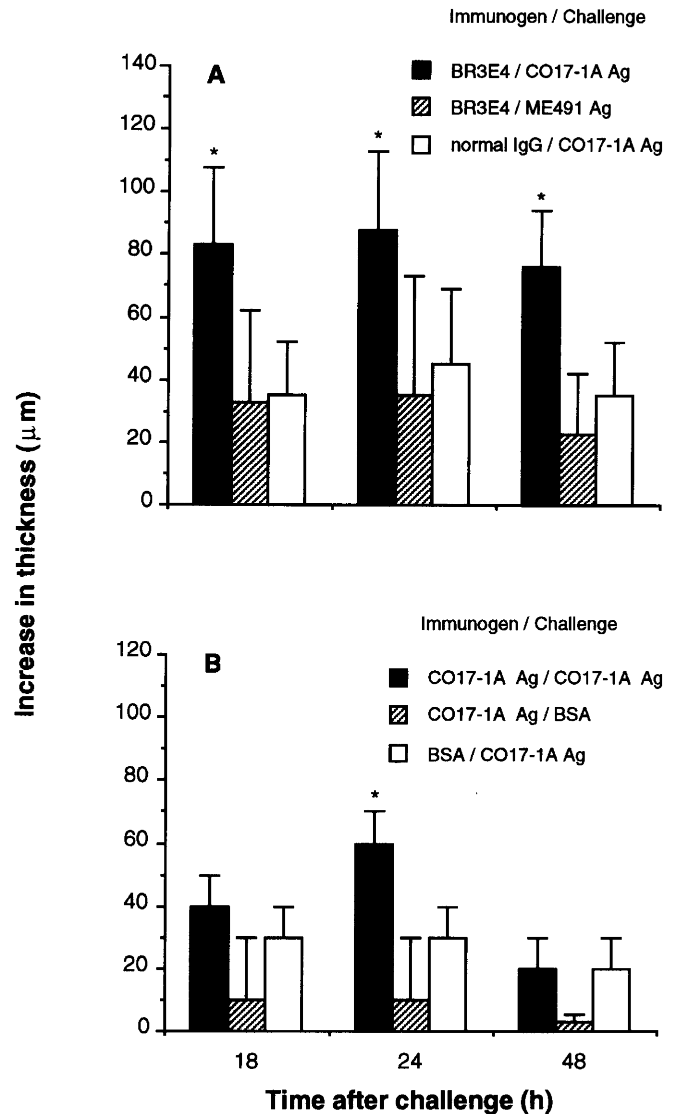

Fig. 6A, B DTH responses of mice immunized with Ab2 BR3E4 (**A**) or GA733-2E (**B**). Mice (four or five per group) were immunized with 100 μg Ab2 BR3E4 or normal rat IgG in CFA s.c. once or with 5 μg alum-precipitated GA733-2E or BSA s.c. three times at 2-week intervals. After 7 days, mice were challenged i.d. with 1 μg GA733-2E, irrelevant ME491 antigen or BSA. The increase in ear thickness was determined after challenge at the various times indicated. Mean ± SD of four or five mice per group. ★ Values significantly different ($P < 0.05$) from each of the two control values obtained at the same assay time

Absence of tumor-protective immunity in mice immunized with Ab2 BR3E4 in CFA/IFA or recombinant GA733 antigen in alum

Murine CRC cell CT26-ALGA710-3H transfectants grown in syngeneic BALB/c mice for 20 weeks expressed the GA733 antigen in vivo, as determined in immunohistoperoxidase assay with polyclonal rabbit antibodies to the antigen. This reaction was specific since normal rabbit IgG did not bind to the tissues, and parental CT26 cells did not bind rabbit antibodies to the GA733 antigen. However, the transfectants did not express the CO17-1A epitope defined by mAb CO17-1A when cultured only in vitro (results not shown). Therefore, tumor-protective effects of Ab2 BR3E4 immunizations could not be tested in this model. The antigen immunization schedule (5 μg alum-precipitated antigen s.c. on days 0, 14, and 28), which induced significant humoral and cellular immune responses in mice [48] (Fig. 6), was used in mice and assessed for protection against a challenge with the transfected murine CRC cells. GA733-2E was unable to protect mice against tumor challenge (not shown).

Discussion

Monoclonal Ab2 BR3E4 might bear the internal image of the CO17-1A epitope since it induces epitope-specific immunity across species barriers, i.e., in a species different from that used for induction of Ab1 [24]. The Ab2 most likely also mimics antigen structurally, since it demonstrated significant amino acid sequence homology with the GA733 antigen [25]. The Ab2 is superior to the polyclonal Ab2 described earlier [19] in its capacity to elicit antigen-specific Ab3 in rabbits. Antigen-binding Ab3 elicited by monoclonal Ab2 BR3E4 were readily detectable in unprocessed sera, whereas the demonstration of such antibodies in sera from rabbits immunized with polyclonal Ab2 required purification of the Ab3 from the sera [19]. Similar results were obtained with monoclonal and polyclonal Ab2 to mAb GA733 [35, 18] and may be explained by the increased homogeneity and specificity of monoclonal compared to polyclonal Ab2 preparations.

The immunogenicity of the GA733 antigen was superior to that of the monoclonal Ab2 BR3E4 in experimental animals, both in the absence (rabbits) and

presence (mice) of normal tissue expression of the antigen homologue. In rabbits, the CO17-1A antigen induced higher titers of specific antibodies at a lower administration frequency than did the Ab2, when both immunogens were precipitated with alum. In mice, alum-precipitated antigen, but not alum-precipitated Ab2, induced specific humoral immunity. The Ab2 had to be administered in a mycobacterial adjuvant (CFA) to elicit antigen-specific humoral immunity in mice. The differences between the immunogenicity of alum-precipitated Ab2 in rabbits and that in mice may be due to differences in the immunological carrier effect of the rat Fc (fragment crystallizing) of Ab2 in the two species [28]. Although Ab2 BR3E4 in CFA/IFA was able to induce antigen-specific humoral immunity in mice, the same Ab2 preparation failed to induce antigen-specific lymphoproliferative responses in mice. However, the latter responses were readily induced by alum-precipitated GA733-2E. On the other hand, the Ab2 in CFA did induce antigen-specific DTH responses in mice and these responses were higher than the responses induced by antigen in alum. Thus, with the exception of DTH responses, all other immune responses we have investigated (cell-binding or antigen-binding antibodies, proliferative lymphocytes) were better in antigen-immunized than in Ab2-immunized mice and rabbits. The explanation for antigen being more immunogenic than Ab2 most likely resides in the expression of multiple rather than single epitopes.

Shearer et al. [44] have compared the immunogenicity and protective activity of recombinant simian-virus (SV)-40-induced large T antigen and an Ab2 mimicking an SV40 epitope in mice. The antigen was consistently superior to the Ab2 in inducing humoral and protective antitumor immunity. SV40-induced antigens are tumor-specific since they are not expressed on normal mouse tissues. Thus, that study compared the immunogenicity of antigen to that of Ab2 in the absence of normal tissue expression and immunological tolerance of the antigen. Our study confirms those observations in the immunologically tolerant host. In contrast, Stein and Söderström [47] reported that Ab2, mimicking a bacterial antigen, primed neonatal (immunologically tolerant) mice for protection against infection with the bacteria, whereas the antigen itself did not. Thus, the outcome of comparative immunizations with antigen and Ab2 in the immunologically tolerant host may depend on the nature of immunological tolerance (adult, as in this study, compared to neonatal tolerance [47]), the nature of Ab2 (Ab2 of the internal antigen image type, as in this study, compared to the most likely regulatory Ab2 [47]) and/or the origin of the antigen or epitope mimicked by Ab2 (self-antigen, as in this study, vs compared to bacterial xenogeneic antigen [47]).

Although we cannot exclude the possibility that the immune responses to the GA733 antigen in mice were directed primarily against the portion (approx. 18% [3]) of the molecule that differs in its sequence from the murine antigen homologue and less against the region that is identical in both species, we have recently successfully immunized mice with the murine antigen homologue precipitated with alum (our unpublished data). Thus, even when the immunogen is identical to the tissue-expressed antigen, alum-precipitated antigen is capable of breaking immunological tolerance.

Clinical trials in cancer patients have already demonstrated induction of immunity to tumor-associated antigens or epitopes, including the CO17-1A epitope, that are also expressed on normal tissues [7, 9, 10, 11, 20, 22, 33, 36, 40]. Induction of immunity was not accompanied by adverse side-effects despite normal tissue expression of the antigens/epitopes. Similarly, induction of immunity against the human GA733 antigen (not shown) or the murine antigen homologue (our unpublished results) in mice was not accompanied by histopathological abnormalities in those organs that express the antigen homologue [51].

GA733-2E antigen, administered as alum precipitate (this study) or with the adjuvants DetoX, interleutin-12, theramide or liposomes (our unpublished data), was unable to protect mice against a challenge with syngeneic CRC transfectants expressing the antigen after cDNA transfer, although the antigen induced specific antibodies and proliferative lymphocyte and DTH responses. These results are reminiscent of the results obtained with an Ab2 (FG1) that mimics the GA733 epitope [35]. This Ab2 also showed excellent immunogenicity but no tumor-protective activity. In contrast, in our recent studies performed with recombinant GA733 antigen (GA733-2 cDNA [50]) expressed in an adenovirus vector, the recombinant virus induced regression of established syngeneic CRC transfectants expressing the human antigen, and protection was mediated by cytotoxic antibodies or proliferative and/or cytolytic T lymphocytes [30, 31]. Thus, presentation of the GA733 antigen by MHC class I molecules of host cells infected with the recombinant viruses [4] might be a prerequisite for this antigen to induce protective antitumor immunity. The question of whether Ab2 BR3E4 expressed in recombinant adenovirus is capable of inducing protective immunity remains unanswered because murine CRC cells do not express the CO17-1A epitope. Since these cells express the epitope defined by mAb GA733, future studies will determine whether Ab2 FG1 mimicking this epitope can induce protective immunity in mice when the Ab2 is expressed in recombinant adenovirus.

Acknowledgements This work was supported by Public Health Service grants CA-43735, CA-10815 and CA-53411 from the National Institutes of Health, and a grant from Ajinomoto Co., Tokyo, Japan. We thank Traci Zell, Margaret Enverso, and Dawei Zhu for excellent technical assistance, and Kristin Smith for editorial assistance.

References

1. Atkinson B, Ernst C, Ghrist BFD, Herlyn M, Blaszczyk M, Herlyn D, Ross AH, Steplewski Z, Koprowski H (1984)

Identification of melanoma-associated antigens using fixed tissue screening of antibodies. Cancer Res 44: 2577
2. Basak SK, Speicher DW, Eck S, Wunner W, Maul G, Simmons M, Herlyn D (1998) Colorectal carcinoma growth and invasion inhibition by CO17-1A/GA733 antigen and its murine homologue. J Natl Cancer Inst 90: 691
3. Bergsagel LP, Victor-Kobrin C, Timblin C, Trepel J, Kuehl WM (1992) A murine cDNA encodes a pan-epithelial glycoprotein that is also expressed on plasma cells. J Immunol 148: 590
4. Berkner KL (1988) Development of adenovirus vectors for the expression of heterologous genes. BioTechnology 6: 616
5. Brattain MG, Strobel-Stevens J, Fine D, Webb M, Sarrif AM (1980) Establishment of mouse colonic carcinoma cell lines with different metastatic properties. Cancer Res 40: 2142
6. Bumol TF, Marder P, DeHerdt SV, Borowitz MJ, Apelgren LD (1988) Characterization of the human tumor and normal tissue reactivity of the KS 1/4 monoclonal antibody. Hybridoma 7: 407
7. Chapman PB, Livingston PO, Morrison ME, Williams L, Houghton AN (1994) Immunization of melanoma patients with anti-idiotypic monoclonal antibody BEC2 (which mimics GD3 ganglioside): pilot trials using no immunological adjuvant. Vaccine Res 3: 59
8. Demetrick DJ, Herlyn D, Tretiak M, Creasey D, Clevers H, Donoso LA, Vennegoor CJGM, Dixon WT, Jerry LM (1992) The ME491 melanoma-associated glycoprotein family. Antigenic identification of the ME491, NKI/C-3, NGA and CD63 proteins. J Natl Cancer Inst 84: 422
9. Denton GWL, Durrant LG, Hardcastle JD, Austin EB, Sewell HF, Robins RA (1994) Clinical outcome of colorectal cancer patients treated with human monoclonal anti-idiotypic antibody. Int J Cancer 57: 10
10. Foon KA, John WJ, Chakraborty M, Sherratt A, Garrison J, Flett M, Bhattacharya-Chatterjee M (1997) Clinical and immune responses in advanced colorectal cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. Clin Cancer Res 3: 1267
11. Foon KA, Sen G, Hutchins L, Kashala OL, Baral R, Banerjee M, Chakraborty M, Garrison J, Reisfeld RA, Bhattacharya-Chatterjee M (1998) Antibody responses in melanoma patients immunized with an anti-idiotype antibody mimicking disialoganglioside GD2. Clin Cancer Res 4: 1117
12. Gottlinger HG, Funke I, Johnson JP, Gokel JM, Riethmuller G (1986) The epithelial cell surface antigen 17-1A, a target for antibody-mediated tumor cell therapy: its biochemical nature, tissue distribution and recognition by different monoclonal antibodies. Int J Cancer 38: 47
13. Herlyn D, Birebent B (1999) Advances in cancer vaccine development. Ann Med 31: 66
14. Herlyn D, Koprowski H (1982) IgG2a monoclonal antibodies inhibit human tumor growth through interaction with effector cells. Proc Natl Acad Sci USA 79: 4761
15. Herlyn M, Steplewski Z, Herlyn D, Koprowski H (1979) Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. Proc Natl Acad Sci USA 76: 1438
16. Herlyn M, Steplewski Z, Herlyn D, Clark WH Jr, Ross AH, Blaszczyk M, Pak KY, Koprowski H (1983) Production and characterization of monoclonal antibodies against human malignant melanoma. Cancer Invest 1: 215
17. Herlyn D, Herlyn M, Ross AH, Ernst C, Atkinson B, Koprowski H (1984) Efficient selection of human tumor growth-inhibiting monoclonal antibodies. J Immunol Methods 73: 157
18. Herlyn D, Ross AH, Koprowski H (1986) Anti-idiotypic antibodies bear the internal image of a human tumor antigen. Science 232: 100
19. Herlyn D, Ross AH, Iliopoulos D, Koprowski H (1987) Induction of specific immunity to human colon carcinoma by anti-idiotype antibodies to monoclonal antibody CO17-1A. Eur J Immunol 17: 1649
20. Herlyn D, Wettendorff M, Schmoll E, Iliopoulos D, Schedel I, Dreikhausen U, Raab R, Ross AH, Jaksche H, Scriba M, Koprowski H (1987) Anti-idiotype immunization of cancer patients: modulation of the immune response. Proc Natl Acad Sci USA 84: 8055
21. Herlyn D, Benden A, Kane M, Somasundaram R, Zaloudik J, Sperlagh M, Marks G, Hart E, Ralph C, Wettendorff M, Mastrangelo M (1991) Anti-idiotypic cancer vaccines: preclinical and clinical studies. In Vivo 5: 615
22. Herlyn D, Harris D, Zaloudik J, Sperlagh M, Maruyama H, Jacob L, Kieny M-P, Scheck S, Somasundaram R, Hart E, Ertl H, Mastrangelo M (1994) Immunomodulatory activity of monoclonal anti-idiotypic antibody to anti-colorectal carcinoma antibody CO17-1A in animals and patients. J Immunother 15: 303
23. Herlyn D, Jacob L, Somasundaram R (1994) Cancer vaccines: preclinical studies. In: Rosenberg SA (ed) Biologic therapy of cancer. Lippincott Philadelphia
24. Herlyn D, Somasundaram R, Li W, Maruyama H (1996) Anti-idiotype cancer vaccines: past and future. Cancer Immunol Immunother 43: 65
25. Herlyn D, Somasundaram R, Jacob L, Li W, Zaloudik J, Maruyama H, Benden A, Harris D, Mastrangelo M (1997) Anti-idiotypic antibodies that mimic the colorectal cancer antigen CO17-1A/GA733: twelve years of pre-clinical and clinical studies. In: Shoenfeld Y, Kennedy R, Ferrone S (eds) Idiotypes in medicine – infections, autoimmunity and cancer. Elsevier Pergamon, Amsterdam, p 477
26. Hruby DE (1990) Vaccinia virus vectors: new strategies for producing recombinant vaccines. Clin Microbiol Rev 3: 153
27. Kasai Y, Herlyn D, Sperlagh M, Maruyama H, Matsushita S, Linnenbach AJ (1992) Molecular cloning of murine monoclonal anti-idiotypic Fab. J Immunol Methods 155: 77
28. Koido T, Scheck S, Herlyn D (1995) Induction of immunity to colon carcinoma antigen CO17-1A by monoclonal anti-idiotype (Ab2): effects of Ab2 fragmentation, carrier and adjuvant. Tumor Targeting 1: 115
29. Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P (1979) Colorectal carcinoma antigens detected by hybridoma antibodies. Somat Cell Genet 5: 957
30. Li WP, Zaloudik J, Gönczöl E, Ricciardi R, Berencsi K, Somasundaram R, Herlyn D (1994) Targeting of the CO17-1A/GA733 antigen in immunotherapy of colorectal carcinoma. Abstract. Cancer vaccines: structural basis for vaccine development. Cancer Research Institute, New York, NY
31. Li W, Berencsi K, Basak S, Somasundaram R, Ricciardi RP, Gönczöl E, Zaloudik J, Linnenbach A, Maruyama H, Miniou P, Herlyn D (1997) Human colorectal cancer (CRC) antigen CO17-1A/GA733 encoded by recombinant adenovirus inhibits growth of established CRC cells in mice. J Immunol 159: 763
32. Litvinov SV, Velders MP, Bakker HAM, Fleuren GJ, Warnaar SO (1994) Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule. J Cell Biol 125: 437
33. Loibner H, Plot R, Rot A, Werner G, Wrann M, Samonigg H, Schmid M, Stoger H, Truschnig M, Herlyn D, Koprowski H (1990) Immunoreactivity of patient with colorectal cancer metastasis after immunization with anti-idiotypes. Lancet 335: 171
34. Mach J-P, Chatal J-F, Lumbroso J-D, Buchegger F, Forni M, Ritschard J, Berche C, Douillard J-Y, Carrel S, Herlyn M, Steplewski Z, Koprowski H (1983) Tumor localization in patients by radiolabeled monoclonal antibodies against colon carcinoma. Cancer Res 43: 5593
35. Maruyama H, Benden A, Zaloudik J, Koido T, Li W, Taupin J-L, Acres B, Somasundaram R, Prewett M, Herlyn D (1996) Monoclonal anti-idiotypic antibody functionally mimics the human gastrointestinal carcinoma epitope GA733. Int J Cancer 65: 547
36. Mittelman A, Chen ZJ, Yang H, Wong GY, Ferrone S (1992) Human high molecular weight melanoma-associated antigen (HMW-MAA) mimicry by mouse anti-idiotypic monoclonal antibody MK2-23: induction of humoral anti-HMW-MAA immunity and prolongation of survival in patients with stage IV melanoma. Proc Natl Acad Sci USA 89: 466

37. Morahan G (1983) A sensitive radioimmunoassay for the detection of monoclonal anti-idiotope antibodies. J Immunol Methods 57: 165
38. Powe J, Herlyn D, Alavi A, Munz D, Steplewski Z, Koprowski H (1985) Radioimmunodetection of human tumor xenografts by monoclonal antibodies correlates with antibody density and affinity. In: Button K, Donato L (eds) Immunoscintigraphy. Gordon and Breach, NY, p 135
39. Riethmüller G, Holz E, Schlimok G, Schmiegel W, Raab R, Höffken K, Gruber R, Funke I, Pichlmaier H, Hirche H, Buggisch P, Witte J, Pichlmayr R (1998) Monoclonal antibody therapy for resected Dukes' C colorectal cancer: seven-year outcome of a multicenter randomized trial. J Clin Oncol 16: 1788
40. Robbins AR, Denton GWL, Hardcastle JD, Austin EB, Baldwin RW, Durrant LG (1991) Antitumor immune response and interleukin 2 production induced in colorectal cancer patients by immunization with human monoclonal anti-idiotypic antibody. Cancer Res 51: 5425
41. Ross AH, Herlyn D, Iliopoulos D, Koprowski H (1986) Isolation and characterization of a carcinoma-associated antigen. Biochem Biophys Res Commun 135: 297
42. Scatchard G (1949) The attraction of proteins for small molecules and ions. Ann NY Acad Sci 51: 660
43. Sears HF, Herlyn DM, Herlyn M, Grotzinger PJ, Steplewski Z, Koprowski H (1981) Ex vivo perfusion of a tumor-containing colon with monoclonal antibody. J Surg Res 31: 145
44. Shearer MH, Bright RK, Kennedy RC (1993) Comparison of humoral immune responses and tumor immunity in mice immunized with recombinant SV40 large tumor antigen and a monoclonal anti-idiotype. Cancer Res 53: 5734
45. Simon B, Podolsky DK, Moldenhauer G, Isselbacher KJ, Gattoni-Celli S, Brand SJ (1990) Epithelial glycoprotein is a member of a family of epithelial cell surface antigens homologous to nidrogen, a matrix adhesion protein. Proc Natl Acad Sci USA 87: 2755
46. Somasundaram R, Zaloudik J, Jacob L, Benden A, Sperlagh M, Hart E, Marks G, Kane M, Mastrangelo MJ, Herlyn D (1995) Induction of T- and B-cell immunity in colon carcinoma patients by anti-idiotypic antibody. J Immunol 155: 3253
47. Stein KE, Söderström T (1984) Neonatal administration of idiotype or antiidiotype primes for protection against Escherichia coli K13 infection in mice. J Exp Med 160: 1001
48. Strassburg CP, Kasai Y, Seng BA, Zaloudik J, Herlyn D, Koprowski H, Linnenbach AJ (1992) Baculovirus recombinant expressing a secreted form of a trans-membrane carcinoma-associated antigen. Cancer Res 52: 815
49. Strnad J, Hamilton AE, Beavers LS, Gamboa GC, Apelgren LD, Taber LD, Sportsman JR, Bumol TF, Sharp JD, Gadski RA (1989) Molecular cloning and characterization of a human adenocarcinoma/epithelial cell surface antigen complementary DNA. Cancer Res 49: 314
50. Szala S, Froehlich M, Scollon M, Kasai Y, Steplewski Z, Koprowski H, Linnenbach A (1990) Molecular cloning of cDNA for the carcinoma-associated antigen GA733-2. Proc Natl Acad Sci USA 87: 3542
51. Zaloudik J, Basak S, Nesbit M, Speicher DW, Wunner WH, Grotkowski CE, Kennedy R, Bergsagel L, Koido T, Herlyn D (1997) Expression of an antigen homologous to the human CO17-1A/GA733 colon cancer antigen in animal tissues. Br J Cancer 76: 909