Phase I Study of Anticolon Cancer Humanized Antibody A33

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ABSTRACT

Purpose: Humanized A33 (huA33; IgG1) monoclonal antibody detects a determinant expressed by 95% of colorectal cancers and can activate immune cytotytic mechanisms. The present study was designed to (a) define the toxicities and maximum tolerated dose of huA33 and (b) determine huA33 immunogenicity.

Experimental Design: Patients (n = 11) with advanced chemotherapy-resistant colorectal cancer received 4-week cycles of huA33 at 10, 25, or 50 mg/m²/week. Serum samples were analyzed using biosensor technology for evidence of human antihuman antibody (HAHA) response.

Results: Eight of 11 patients developed a HAHA response. Significant toxicity was limited to four patients who developed high HAHA titers. In two of these cases, infusion-related reactions such as fevers, rigors, facial flushing, and changes in blood pressure were observed, whereas in the other two cases, toxicity consisted of skin rash, fever, or myalgia. Of three patients who remained HAHA negative, one achieved a radiographic partial response, with reduction of serum carcinoembryonic antigen from 80 to 3 ng/mL. Four patients had radiographic evidence of stable disease (2, 4, 6, and 12 months), with significant reductions (>25%) in serum carcinoembryonic antigen levels in two cases.

Conclusions: The complementarity-determining region-grafted huA33 antibody is immunogenic in the majority of colon cancer patients (73%). HAHA activity can be measured reproducibly and quantitatively by BIACORE analysis. Whereas the huA33 construct tested here may be too immunogenic for further clinical development, the antitumor effects observed in the absence of antibody-mediated toxicity and in this heavily pretreated patient population warrant clinical testing of other IgG1 humanized versions of A33 antibody.

INTRODUCTION

The original mAb A33 is a murine IgG2a that has undergone extensive preclinical analysis (1–8) and a series of pilot Phase I localization-radioimmunotherapy studies from 1991–1995 (9–11). Immunohistochemical testing of a large panel of normal and malignant tissues has shown A33 antigen expression to be restricted to the epithelia of the lower gastrointestinal tract and to carcinoma lesions that originate from the rectal and colonic mucosa (2). Despite limited normal tissue reactivity, this antigenic system was selected as a target for clinical studies because the A33 antigen is expressed homogeneously and at high levels in 95% of cases of metastatic colorectal cancers (2).

Although much has been learned regarding the biochemical, immunological, and molecular biology of the A33 antigen, the function of this molecule remains unknown. The A33 antigen is a Mr 43,000 glycoprotein that has been sequenced; its cDNA has been cloned (12–15), and posttranslational modifications have been characterized (15). Biochemical and fluorescence microscopy experiments have shown that a portion of cell-bound mAb A33 is internalized into cytoplasmic vesicles (1). In general, antibodies targeting cell surface antigens, which are internalized into pathways that lead to the lysosomal vesicles, have been found to undergo rapid catabolism (16, 17). In contrast, cell-bound mAb A33 represents a stable pool of antibody in equilibrium between the cell surface and macropinosomal or endosomal compartments (1). The clinical observation that radioiodinated mAb A33 is retained in tumor tissues for up to 6 weeks has led to the hypothesis that this retention is a consequence of the unique cell trafficking pathways of this antibody, which only slowly enters the lysosomal compartment (1, 10).

In our initial clinical imaging and biopsy-based biodistribution studies using the mouse A33 antibody, we observed specific targeting to antigen-positive tumor tissues in 95% of patients (9–11). Whereas the only other tissue that concentrated the radioisotope was bowel, clearance from the gastrointestinal tract was much more rapid than that from tumor tissue, so that at the end of the imaging period, only the tumors were visualized (11). These localization characteristics led us to a Phase I 131I-labeled mAb A33 therapy trial that demonstrated bone marrow as the dose-limiting organ toxicity (9). As the colon expresses A33 antigen, gastrointestinal toxicity was monitored...
closely and found to be minimal at the MTD for the bone marrow (80 mCi/m²). Antitumor effects were observed in 5 of 23 assessable patients, despite the fact that only a single dose could be administered due to the development of an antinuclide response (human antimouse antibody) in all patients (9). Based on the unique internalization pathway of mAb A33 and its retention in tumor cells, we also examined whether a low-energy electron-emitting radionuclide, such as 125I, might have therapeutic advantages over high-energy β emitters such as 131I (1, 10). This concept was supported by therapy experiments in a nude mouse model, which demonstrated complete ablation of tumors with nontoxic doses of 125I-mAb A33 (8). This clinical study confirmed the longer retention of 125I-mAb A33 in tumor sites when compared with the uptake by the colon, and external imaging of the 125I was possible even 6 weeks after antibody administration (10). Whereas the radiotherapeutic doses tested did not approach the MTD of bone marrow or other normal tissues, plasma levels of 125I-mAb A33 reached cytotoxic levels for colon cancer cells based on in vitro assays. Minor antitumor effects were observed in 4 of 22 patients (10). Of note was the observation that additional major responses were observed in a subgroup of patients who were treated with a specific chemotherapy regimen (10) after treatment with 125I-mAb A33. This finding is consistent with results in a mouse model indicating synergistic antitumor effects between radiolabeled A33 antibody and chemotherapy (3).

These preliminary studies with mAb A33 have defined the targeting capability of this antigenic system as well as its limitations. However, full characterization of the therapeutic potential of the mouse-derived A33 antibody could not be accomplished due to the inability to effectively retreat patients with an immunogenic foreign protein. Thus, a fully humanized CDR-grafted IgG1 was developed (18, 19) and evaluated in the current report. This IgG1 humanized antibody has the added advantage of being able to direct cell-mediated immune lysis of human colon cancer cell lines in vitro. As a consequence of this cytotoxic capability, normal antigen-positive tissue toxicity was monitored closely in this study.

MATERIALS AND METHODS

Production and Purification of huA33 Antibody. huA33, a fully humanized IgG1 mAb, was derived from murine A33 antibody by CDR grafting (18). The antibody was expressed in NSO cells, and culture supernatant was produced in bioreactors by Celltech Limited (Berkshire, United Kingdom) and concentrated. huA33 was purified from NSO supernatant at the New York Branch of the Ludwig Institute for Cancer Research at MSKCC, using a three-step chromatography process: Q-Sepharose anion-exchange; protein A affinity; and S-Sepharose cation exchange chromatography. Virus inactivation was achieved by exposure of the protein A elute to pH 3.0 at room temperature for 30 min. The antibody was formulated in PBS [0.05 M sodium phosphate and 0.15 M sodium chloride (pH 7.0)], sterile filtered, and stored at −70°C. The purified huA33 did not form aggregates, did not contain detectable levels of protein A, and retained the original binding specificity of the murine A33 antibody (20).

Patient Selection and Eligibility. Histology slides from all patients were reviewed by the Department of Pathology at MSKCC and confirmed to be colorectal carcinoma. Eligibility criteria were as follows: patients had to be at least 18 years of age; have unresectable stage IV disease; and have tumors refractory to standard chemotherapy and measurable or evaluable by conventional imaging. Patients had to be off prior therapy for at least 4 weeks, be ambulatory with a Karnofsky performance status of 70 or greater, and have an estimated survival of at least 6 weeks. Laboratory requirements included serum creatinine < 2 mg/100 ml, serum bilirubin < 2 mg/100 ml, granulocyte count > 1,500/mm³, platelet count > 100,000/mm³, and prothrombin time < 1.3 × control. All patients had to be capable of giving written informed consent according to institutional and federal guidelines. Patients with the following criteria were excluded from the study: clinically significant cardiac disease (New York Heart Association class III/IV); infections requiring antibiotics; illnesses requiring the use of steroids or other anti-inflammatory reagents; clinical evidence of central nervous system tumor involvement; history of prior administration of mouse-derived antibodies or protein constructs or human anti-mouse antibody serum reactivity; and positive pregnancy test or lactation.

Study Design/Administration of huA33. The study protocol was reviewed and approved by the Institutional Review Board at MSKCC (Protocol 94-100), and the study was conducted under a United States Food and Drug Administration Investigational New Drug Application (BB-IND #6084). The study was designed with two major objectives: (a) to define the toxicities and MTD of huA33 administered in weekly doses; and (b) to determine whether huA33 is immunogenic and characterize the anti-idiotypic response and toxicities associated with the development of anti-huA33 Ig (human antihuman Ig antibody, HAHA).

Patients were monitored with weekly complete blood counts, weekly tests for occult blood in stools, and liver function and renal function tests every 4 weeks. CT scan evaluation of response was performed at least every 9 weeks. We had previously shown that upon retreatment, the mouse A33 antibody was cleared rapidly and prevented from targeting colon cancer in the presence of human antihuA33 antibody (9–11). Thus, in addition to directly monitoring HAHA levels using biosensor technology (BIACORE), we also measured A33 antigen-reactive huA33 in serum (by ELISA) as another indicator of anti-huA33 activity after treatment. Because clinical toxicities directly related to the effects of huA33 binding to cell surface A33 antigen would be masked by the presence of neutralizing antibodies (HAHA), it was critical to detect the presence of even low titers of anti-huA33 activity. Blood samples were collected every week, before and after antibody infusion, to monitor HAHA activity. Thus, the observed toxicities could be correlated to the presence or absence of HAHA throughout the treatment course. Doses (10–50 mg/m²/week) and schedule (weekly doses) were selected based on the amount of clinical grade huA33 available and the preliminary clearance rates of radiolabeled huA33 antibody.

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria. Dose-limiting toxicity was defined as follows: any grade 2 or greater allergic reaction; any...
grade 4 nonhematological toxicity; any grade 4 WBC toxicity lasting >1 week (2 consecutive weeks); or any grade 4 platelet count toxicity requiring platelet transfusion. To be dose-limiting, an adverse event had to have been judged by the principal investigator to be possibly related to the investigational agents. The MTD was defined as the dose level immediately below the dose level inducing dose-limiting toxicity in two patients (of three to six patients tested). Standard criteria for response to treatment were based on radiological examination before and after treatment cycles (2 cycles or 9 weeks), and response was rated as complete (disappearance of all lesions), partial (50% decrease or >25% increase in the sum of the products of the perpendicular diameters), stable (<50% decrease or <25% increase in the sum of the products of the perpendicular diameters), or progression (>25% increase or new lesions). Serum CEA levels were monitored every 5 weeks.

The antibody was diluted in 100–150 ml of physiological saline containing 5% human serum albumin and infused through a line containing normal saline at a rate of approximately 1 mg/min. All patients were treated in the outpatient facility, and all infusion-related toxicities had to be resolved before discharge. Patients who could not be treated as outpatients were removed from the study.

**Immune Response to huA33 and Pharmacokinetics.** Induction of an antibody response to huA33 (HAHA) was monitored by surface plasmon resonance technology using a BIACORE 2000 instrument (21). This methodology allows direct measurement of specific antihuman Ig antibodies in sera of patients, which bind to huA33 in a label-free manner in real time. huA33 and control antibodies were immobilized by amine coupling to CM5 biosensor microchips. Control antibodies included murine anti-A33 mAbs, A33 (IgG2a), and 100.310 (IgG2a; Ref. 11). SK10B, a humanized IgG1 of the same allootype as huA33, and a humanized IgG4 antibody, SK10C, were provided by Celltech. Goat antihuman IgG, goat antihuman IgM, and goat antimonuse IgG were obtained from Sigma Chemical Co. (St. Louis, MO). mAbs against human IgG, IgE, and IgM were from Zymed Laboratories, Inc. (San Francisco, CA). Antibodies against human IgG1, IgG2, IgG3, and IgG4 were from Southern Biotechnologies (Birmingham, AL). Diluted patient serum (1:100) was passed over the chip, and alterations in the refractive index were recorded as the relative RUs (21).

Controls included pretreatment of serum samples with protein G-Sepharose to remove IgG and blocking with huA33 or control antibodies before BIACORE analysis (21). huA33 serum levels were assayed by ELISA, using recombinant A33 antigen-coated plates incubated with serial dilutions of serum samples. Bound huA33 was quantitated with alkaline phosphatase-coupled goat antihuman IgG. Attophos (JBL, San Luis Obispo, CA) was used as a substrate, and fluorescence was measured in a Cytofluor 2350 reader (Millipore, Bedford, MA).

**RESULTS**

**Patient Characteristics**

Twelve patients were registered into this study. Characteristics of the 11 evaluable patients are listed in Table 1. All patients had tumors resistant to conventional chemotherapy consisting of a fluorouracil-based regimen and received a mean of 3.3 chemotherapeutic agents before protocol entry. All patients had stage IV disease; two patients had known metastatic colorectal cancer limited to one site (lung), two patients had two sites of disease (pelvic/lung and liver/lung), and seven patients had three or more sites of disease. Review of tissue sections in the Department of Pathology, MSKCC confirmed that all patients had colorectal cancer. Because the antigen is not detectable after formalin fixation using mAb A33, tumor typing for A33 antigen expression was not performed. Because the A33 antigen is expressed in >95% of colorectal cancers, requiring additional fresh-frozen biopsy specimens specifically for A33 antigen typing was not justified.

All patients who received at least one full dose of huA33 were considered evaluable for toxicity (n = 11). One patient with underlying hypertension and coronary artery disease could not tolerate the saline/human serum albumin infusion and received <10% of her first dose; she was removed from the study and excluded from the analysis.

**Toxicity**

**Induction of Anti-huA33 Activity in Serum.** Blood samples collected before and after each huA33 treatment were monitored for anti-huA33 reactivity. Two patterns of reactivity (type I and type II) emerged from this serological analysis, and these data have been reported in detail elsewhere (21). Patients

<table>
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<th>Patient no.</th>
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<th>Site</th>
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<th>Serum CEA (ng/ml)</th>
<th>LDH (units/dl)</th>
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</table>

* Site of primary disease: A, ascending colon; D, descending colon; R, rectum; S, sigmoid colon.

* HAHA response; II, type II HAHA response.

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with a type II response developed anti-huA33 antibodies with progressively increasing titers. This pattern has been the general experience with HAHA activities. Patients with a type I response to huA33 developed a limited anti-huA33 response usually by week 2 or 3 and had stable levels of activity, that resolved, in most cases, by week 7 or 8 of treatment (tolerance). Induction of reactivity was scored as type II [persistently increasing titers (n = 3); patients 5, 9, and 10], type I [stable or transient levels of anti-huA33 activity (n = 5); patients 2, 4, 6, 8, and 11], or no detectable reactivity (n = 3; patients 1, 3, and 7; Table 1; Fig. 1). Patient 4 had modestly high titers of anti-huA33 activity, but the titers stabilized, and she was therefore designated as a type I pattern. Serum reactivity was shown to be specific for huA33-variable regions or CDRs by preincubation of serum samples with either huA33, mouse mAb A33, or control humanized or mouse antibodies. No IgG1 constant region (allootypic) reactivity was demonstrated. There did not appear to be a clear correlation between induction of anti-huA33 and antibody dose level because HAHA occurred in all dose levels; however, the highest titer was in a patient at the highest antibody dose level (patient 9). The duration of treatment did not appear to be a factor because patients developed type II HAHA titers as early as the 4th week of treatment (patient 9) or only after 5 months (patient 10), whereas one patient was treated for almost 4 years without evidence of HAHA (patient 3). Low HAHA titers of the type I pattern appeared earlier than those of type II and were detectable at week 2 in some cases (patients 2, 6, and 8).

Serum samples from four patients (patients 4, 5, 9, and 10) with strong anti-huA33 serum titers, type I or type II patterns, were shown to have clinically significant huA33 binding activities by two assays. In the first assay (BIACORE), anti-huA33 activity was measured in percentage response units of post- versus pretreatment serum samples (Fig. 2). In one case, HAHA was shown to decrease only modestly, even after an infusion of a 100-mg dose (50 mg/m² dose level) of huA33 (Fig. 2, patient 9). Thus, there were sufficiently high levels of anti-huA33 activity in the blood so that an administration of 100 mg of huA33 could not completely neutralize the HAHA activity. The second assay measured serum huA33 antibody binding to solid phase A33 antigen from blood samples collected after each antibody infusion. The results indicate that once the patients developed detectable (either type II or type I or high or low titer response) anti-huA33 activity by biosensor methodology (n = 8), they cleared or neutralized virtually all or a substantial portion of serum huA33 binding activity to antigen (data not shown).

Clinical toxicity correlates of these laboratory results indicated that two of four patients (patients 9 and 10) with high anti-huA33 activity had acute infusion-related toxicities such as fever, rigors, facial flushing, nausea with vomiting, and changes in blood pressure (grade 1–2) resulting in discontinuation of treatment (Table 2). In contrast, toxicities in the other patient with a type II pattern of HAHA and in the five patients with a type I pattern of HAHA activity consisted of persistent erythematous rashes, myalgias, and fevers in two of these patients (patients 4 and 5) and no symptoms in the remainder (patients 2, 6, 8, and 11). The four patients (patients 4, 5, 9, and 10) with symptoms had the absolute highest titers by BIACORE analysis, and these symptoms only occurred when BIACORE measurements of HAHA exceeded 100 response units, suggesting that symptoms correlate quantitatively with BIACORE results.

Further characterization of the serum anti-huA33 activity of these patients indicated that HAHA consisted of an IgG response, without evidence for significant IgM activity and no detectable IgE anti-huA33 antibodies. This was the case for both type I and type II patterns. Of note is that the response detected at 2 weeks consisted of IgG antibodies, which was consistent with a memory response rather than a primary immunization. An anti-HAHA response or an idiotope network antibody 3 response, reactive with A33 antigen, could not be detected. The four patients with the highest HAHA levels did not have IgE anti-huA33 or eosinophilia. One patient without HAHA activity received only 4 weeks of antibody (1 cycle). Thus, of the 10 patients receiving more than 4 weeks of treatment, 8 patients (80%) developed HAHA, and 4 patients (40%) had clinical
symptoms. Although tolerance can be achieved with continued administration of huA33 in patients with low levels of HAHA, in practical terms, antibody treatment during a type I response may have no benefit for the patient because antibody-antigen binding activity is blocked. The fine specificity analysis (epitope mapping) of the induced anti-A33 activity is described in Ref. 21.

Adverse Reactions to huA33. Eight patients (patients 1, 2, 3, 5, 6, 7, 9, and 10) reported one or more of the following symptoms (mild rhinorrhea, cough, periorbital fullness, or headache) occurring by the end of the infusion or within a few hours thereafter. These symptoms, which commonly started with the first infusion and always resolved by the next day, occurred in patients who never developed HAHA or before the development of HAHA and thus are attributable to the effects of huA33 alone (Table 2).

Two patients (patients 3 and 7), one each from the first and second dose levels, developed eosinophilia after 24 and 20 months of treatment, respectively. Eosinophil counts ranged from 15–25% (normal up to 7%) of WBC counts, whereas other blood elements remained unchanged or within normal limits. At the time the eosinophilia was first noted, patient 3 also had intermittent episodes of diarrhea (grade 2), and patient 7 developed a pruritic erythematous rash with a minor urticarial component (grade 2). Colonoscopy of patient 3 did not reveal any gross pathology, and a blind biopsy demonstrated only normal mucosa. Eosinophilia resolved spontaneously in patient 3 when treatment was withheld for 5 weeks. A skin biopsy of a rash site of patient 7 showed an intense perivascular eosinophilic infiltration. Her antibody treatments were withheld, and she received a short course of prednisone (15 mg/day) with resolution of her rash and blood eosinophilia 4 weeks later (grade 3). Neither patient 3 nor patient 7 had evidence of HAHA during the time when eosinophilia was present, and IgE anti-huA33 activity was specifically excluded.

At the third dose level, patient 9 had no adverse events until, beginning with the fifth treatment, she developed infusion-related symptoms consisting of nausea (grade 2), emesis (grade 1), hypotension (grade 1), and fever and rigors (grade 2), which were controlled with Demerol and acetaminophen. On week 8, the patient complained of arthralgias (arrow), and upon treatment she developed a grade 1 decrease in blood pressure, and her treatments were discontinued. Corresponding laboratory values are consistent with a cell lysis syndrome with elevated uric acid and a 10-fold increase in LDH.
after antibody treatments, and thus the antibody treatments may have contributed to an underlying radiation-induced functional bowel disorder. A decrease of her dose from 50 to 10 mg/m$^2$ did not affect the frequency of her symptoms. The patient underwent a surgical procedure in which the segment of involved bowel was resected. Pathology revealed changes consistent with radiation enteritis, without evidence for antibody effects. However, given the antibody’s known targeting to normal bowel and the temporal relationship to the antibody infusions, bowel dysfunction was felt to be reactivated by the huA33 treatments in a fashion similar to chemotherapy induced radiation recall reactions.

Patients had stools monitored for occult blood, and no episodes of antibody-induced bowel bleeding were documented. Diarrhea was a common preexisting condition, but no grade 3 or 4 toxicity was encountered in the huA33 doses examined here, although two patients (patients 9 and 10) at the highest antibody dose level had a low-grade increase in frequency of bowel movements and abdominal pains related to huA33 treatment.

Patient accrual was discontinued before establishing a MTD unrelated to HAHA-associated toxicities, due to the immunogenicity of huA33 (73% of patients) and the inability to give repeated treatments and because all three patients at the highest dose level developed HAHA. Because HAHA activity was not clearly huA33 dose dependent, a MTD could not be assigned to HAHA toxicities. Nevertheless, the most severe HAHA-associated toxicities occurred in the two patients (patients 9 and 10) treated at the highest dose level. These data emphasize the potential toxicity of HAHA-related IgG1 immune complexes.

Responses. All patients had measurable disease and were evaluated for response by CT scan every two cycles (9 weeks), except for patients 1 and 9 (CT scan done on week 5 only). Patient 3 had a partial response of lung and liver metastases for 16 months (Fig. 4). huA33 treatments were discontinued after 1 year, at which point her serum CEA had been normal for 6 months (Fig. 5), and all lesions had disappeared except for a single residual defect in the liver. While off treatment, her pulmonary metastases recurred, and she was restarted on huA33. She had stable disease for another 32 months, eventually progressing in the lymph nodes.

An additional four patients (patients 5, 6, 7, and 10) had evidence of stable disease by CT scan (week 9), with two patients (patients 7 and 10) having reduced serum CEA levels (>25%). Patients 5, 6, 7, and 10 had stable disease for 4, 2, 12, and 6 months, respectively. Patient 7 had stable disease for 12 months when she underwent a surgical debulking procedure. The stable disease observed on CT scan and the modest serum CEA declines were observed only before development of HAHA (patients 5, 6, and 10) or in the absence of HAHA (patient 7). Stabilization of disease was not seen in the presence of HAHA. Four patients had progression of disease on CT scan at week 9.

**DISCUSSION**

The present study indicates that huA33, at the doses examined, induces little toxicity by direct effects of the antibody targeting to antigen in normal tissue. However, the neutralization of huA33 by HAHA may block the complete manifestation of toxicity. All but three patients developed HAHA, and one of these three patients was treated for only 4 weeks. Therefore, a complete evaluation of huA33 as an immunotherapeutic agent must await construction of a nonimmunogenic form of this antibody. Based on the clinical targeting data available for the A33 antibody, we expect that, in the absence of immunogenicity, dose escalation would be limited by bowel toxicity (9–11). Despite the limitations of the current study, it provides preliminary evidence of antitumor activity in a group of heavily pretreated patients at doses below the level where significant bowel toxicity would be observed. One patient had a major response of liver and lung disease, and four patients had stabilization of disease by CT scan. The patient with the major response was one of three patients who did not develop HAHA. The patient with the longest-lasting stable disease was also one of the three who did not develop HAHA. One patient with stable disease and decreasing serum CEA levels subsequently devel-
oped type II HAHA and was removed from the study. No radiological evidence of response or declining serum CEA levels were noted while patients had HAHA. Thus, in addition to blocking tumor targeting of huA33, HAHA is also responsible for abrogating the antitumor activity of the antibody.

The mechanism by which huA33 exerts its antitumor activity is believed to be via activation of immunological effector functions. huA33 has no direct growth-inhibitory or cytotoxic effect on colon cancer cell lines in vitro, and the function of the antigen is unknown (3–5). Human IgG1 antibodies in general have the potential to direct cellular and complement-mediated cytotoxicity, and it was for this reason that we selected IgG1 as the isotype of huA33 for clinic therapy studies (22). As we and others have reported previously (23), critical factors for immune-directed tumor lysis include antibody isotype, cell surface antigen quantity, and density. Quantitative measurements of mAb A33 binding to cell surface A33 antigen on colon cancer cell lines predicted the immune-mediated lytic capability of the humanized version (1). Without tissue biopsies from regressing tumors, it is difficult to document that this mechanism is active in patients. Experience with humanized IgG1 antibodies as mediators of immune effector function in patients with solid tumors has been limited thus far. However, the inherent potential of IgG1 antibodies for activation of immune-mediated effects, such as antibody-dependent cell-mediated cytotoxicity, human complement lysis, opsonization, and induction of targeted inflammation, may render IgG1 isotype treatment a new potent antitumor therapeutic modality.

Studies of the IgG1 anti-p185HER2 antibody (Herceptin) clearly show antitumor activity in selected breast cancer patients whose tumors express high levels of antigen (24). Whether clinical antitumor activity is due to a growth factor-inhibitory effect, Herceptin’s ability to mediate immune-cellular cytotoxicity, or a combination of these two mechanisms is not clear (25). When Herceptin was first engineered, consideration was given to capture both of these activities (cell growth inhibition and antibody-dependent cell-mediated cytotoxicity; Ref. 26); however, from a mechanistic point of view, it is not possible at present to separate these effects in the clinic. Similar considerations apply to the anti-epidermal growth factor receptor antibodies. However, in this case, clinical studies are proceeding, evaluating antibodies with (C225 and IgG1) or without (ABX-EGF and IgG2) immune capabilities or drugs that specifically interrupt the signaling pathways (27, 28). Results in animal studies suggest that immune mechanisms are not required for antitumor activity (29), and clinical studies that are under way will provide important information with regard to sorting out the relative contributions of each antitumor mechanism. Thus, we will be able to distinguish whether the immune effects of antibody add to the antitumor activity of growth factor signal blockade. Another antigenic system, the Lewis Y epitope found on glycoproteins and glycolipids, is expressed at high levels in a large number of solid tumor types, and new humanized anti-Lewis Y IgG1 antibodies are strong mediators of immune-directed lysis (30, 31). Preliminary clinical reports of the Phase I trial of a humanized IgG1 antibody to the 17.1A antigen (anti-EGF40) indicated that toxicity in antigen-expressing pancreatic ducts and bowel may be dose-limiting (32). This is in striking contrast to the experience with the widely used mouse antibody to the 17.1A antigen, demonstrating the potency of the human IgG1 constructs when compared with mouse antibody (32, 33). Even among the human IgG isotypes, there may be subtle differences in effector functions using IgG1 or IgG3 or combinations of these isotypes, and these differences need to be explored (34).

The two patterns of HAHA activity described here for huA33 have been confirmed by analysis of a larger population of patients treated with this antibody (21). Of note is that in the current study, three patients had a type II pattern of HAHA response. These patients had a classical response to an immunogen (huA33), with increasing titers as exposure to antigen continued. Considering the potential toxicity of continued huA33 treatments in patients with type II HAHA responses, this rate of immunogenicity [3 of 11 (27%)] is too high for the general use of this antibody. In addition, five patients had the more unusual HAHA response, a type I pattern, developing a limited response that in some cases resolved (tolerance). Because these HAHA activities occurred early (as soon as week 2), these are not consistent with primary immunological responses but rather secondary responses and we speculate that the huA33 contains a determinant that mimics a common immunogen. Thus, the unexpected high rate of HAHA activity is due to a chance cross-reactivity between one or more epitopes in the variable portion of the huA33 and a common immunogen. To our knowledge, huA33 may be the only such example of an antibody inducing a type I immunogenicity pattern, but this needs further examination. Type I HAHA activities may be missed unless sensitive assays are used, and it may be an important mechanism of resistance to antibody-based therapies regardless of the antitumor mechanism involved. Expectations that the humanization process would be successful for each new reagent was perhaps unrealistic, given the complexity of the engineering required to maintain specific antigen binding. The current study illustrates that in some cases, clinically significant immunogenicity can be induced despite the humanization process.

Due to the binding of huA33 to normal bowel, gastrointestinal symptoms were monitored closely. No occult bleeding was detected. Diarrhea was more difficult to evaluate. Due to the effects of their prior treatments, 6 of 11 patients required antidiarrheal medication at some point in the 30-day period before starting the protocol. However, two of the three patients (patients 9 and 10) at the highest dose level developed increased frequency of bowel movements associated with grade 2 abdominal pains, clearly related to the antibody administration. Of note was that this toxicity occurred only 24–48 h after antibody treatment. Another patient (patient 3) developed significant diarrhea (grade 2) after 1 year of treatments, but only in conjunction with the onset of eosinophilia. When the huA33 treatments were temporarily discontinued, eosinophilia and diarrhea resolved.

Three unusual clinical observations were made during this study that may be of more general relevance regarding immunotherapy with humanized IgG1 antibodies. First, the renal toxicity induced in patient 9, in the setting of continued treatment with huA33 and rising HAHA activity, no doubt led to increasing levels of circulating immune complexes. Because both the immune complexes and hyperuricemia induced in this patient are toxic to the kidneys, the reversal of renal dysfunction...
was particularly difficult in the presence of these two factors. Second, the hyperuricemia of patient 9 appeared to be part of a cell lysis syndrome because it occurred with a 10-fold increase of LDH and minor changes in phosphate and calcium. Hyperkalemia was not a prominent feature because the patient was on furosemide. This patient had a large volume of tumor, and although some rapid tumor lysis may have occurred, the patient remained with a significant amount of active disease. This partial cell lysis may be a manifestation of heterogeneity of antigen expression within the tumor leading to rapid lysis of a subset of colon cancer cells. Alternatively, the cell lysis may have involved normal A33 antigen-expressing cells or nonspecific toxicity due to immune complexes. The third observation regarding reactivation of symptoms in patient 10, with a pathologic diagnosis of radiation enteritis, indicates that human IgG1 binding to normal tissue in the area of prior radiotherapy may reinduce or exacerbate tissue radiotoxicity. The interaction between chemotherapy and radiotherapy is well known. Radiation recall reactions have the potential to cause severe tissue damage. In the case described, retreatment with antibody reinduced a defined radiation-related bowel dysfunction. The corollary to this observation is that there might be additive antitumor effects from combining radiotherapy with the immune effector functions of human IgG1 antibodies.

We had reported previously (10) that a subset of patients with tumors refractory to chemotherapy who were treated with a specific drug regimen after a single dose of radiolabeled mAb A33 had significant responses. We investigated these effects in a mouse model and found that there is synergy between the antitumor activity of 5-fluorouracil and 131I-mAb A33 (3). Similar observations have now been made with some chemotherapeutic agents when they are administered after completing treatment with huA33 without a radiolabel. The significance of these observations will be better defined in subsequent studies designed to investigate the interaction between immunotherapy and chemotherapy (35). The molecular mechanisms critical for the enhancement of immune-directed lysis of tumor cells by chemotherapeutic regimens have been studied since the original observations focused on lipid synthesis (36, 37). Whereas some chemotherapeutic agents have been reported to show synergy with complement and antibody-dependent cellular toxicity, development of relevant assays to identify the most promising drug-antibody combinations and to define the molecular mechanisms critical for this interaction remains of primary importance. Studies have demonstrated that Herceptin has additive or synergistic antitumor effects when used in combination with chemotherapy (38). In this case, the dual activities of Herceptin (cytostatic effects and immune effector function) may both be critical for the enhanced antitumor responses observed when antibody is used in conjunction with chemotherapy in patients (25, 39). Studies with rituximab have also supported the role for an enhancing effect of chemotherapy on immune-mediated killing of tumor cells (40).

Two patients (patients 3 and 9) with evidence of antitumor effects shared a characteristic, which may indicate that a certain level of autoimmune reactivity may be required for antibody-based therapy to be fully effective in the clinic. Both patients had evidence of preexisting autoimmunity. Patient 3 had a diagnosis of polyarthritis rheumatica, and patient 9 had myositis with elevated creatine phosphokinase serum levels and muscle weakness. The evidence of immune-mediated normal tissue dysfunction in these patients may indicate preexisting dominance of activation Fc receptors (41). Identifying biological factors that modulate antibody-directed immune killing in patients is critical to increase the response rate of IgG1 therapeutic antibodies.

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