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Therapy of Patients with Malignant Melanoma Using a Monoclonal Antimelanoma Antibody-Ricin A Chain Immunotoxin[1]

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ABSTRACT

We conducted a trial of a murine monoclonal antimelanoma antibody-ricin A chain immunotoxin (XOMAZYME-MEL) in 22 patients with metastatic malignant melanoma. The dose of immunotoxin administered ranged from 0.01 mg/kg daily for 5 days to 1 mg/kg daily for 4 days (total dose: 3.2 to 300 mg). Side effects observed in most patients were a transient fall in serum albumin with an associated fall in serum protein, weight gain, and fluid shifts resulting in edema. In addition, patients experienced mild to moderate malaise, fatigue, myalgia, decrease in appetite, and fevers. There was a transient decrease in voltage on electrocardiograms without clinical symptoms, change in serial echocardiograms or elevation of creatine phosphokinase MB isozyme levels. Symptoms consistent with mild allergic reactions were observed in three patients. The side effects were related to the dose of immunotoxin administered and were generally transient and reversible. Encouraging clinical results were observed, even after a single course of a low dose of immunotoxin. In addition, localization of antibody and A chain to sites of metastatic disease was demonstrated by immunoperoxidase staining of biopsy specimens. Additional studies are being conducted to continue the evaluation of safety and efficacy of immunotoxin therapy for malignancy.

INTRODUCTION

The technology permitting the development of cultures of fused cells secreting antibody of predefined specificity was first described in 1975 (2). Since then, hybridoma technology has developed rapidly and has permitted the production of monoclonal antibodies for a variety of purposes, including the diagnosis and therapy of malignancy in humans.

The first attempts to apply this technology in the therapy of malignancy involved the use of unmodified monoclonal antibodies (3). The toxic effects encountered from the injection of unmodified monoclonal antibodies in human subjects have generally been minimal. There has also been little evidence of clinical benefit to cancer patients, even when the antibodies were administered in massive doses.

In a single, widely cited case, a dramatic, prolonged clinical response was observed in a patient with B-cell lymphoma given an anti-idiotype antibody (4). In a subsequent study, this group reported objective incomplete remissions which were of relatively short duration in 5 of 10 additional patients (5). In other reports, only a transient decrease in circulating cells was found following treatment with anti-idiotype antibody (6) or antibodies reactive with T-cells (7, 8) with no apparent therapeutic benefit. In patients with malignant melanoma, injection of unmodified antimelanoma antibody 9.2.27 resulted in localization of the antibody in tumor sites, as determined by immunoperoxidase staining, but no clinical benefit (9). In one study, encouraging results were obtained in patients with malignant melanoma using the anti-ganglioside antibody R24 (10).

We anticipated that unmodified monoclonal antibodies, in general, would not be of significant therapeutic benefit to patients with solid tumors. Unmodified antibodies may kill target cells via complement-mediated cytotoxicity or antibody dependent cell-mediated cytotoxicity, both of which are indirect and require involvement of the host immune system. Monoclonal antibodies can also be used to target cytotoxic agents to tumor cells for direct killing. Such an approach has the advantage of increasing selective tumoricidal activity and decreasing the systemic toxicity of these cytotoxic agents. Studies utilizing currently available chemotherapeutic agents conjugated with monoclonal antibodies thus far have demonstrated relatively low cytotoxic activity (11). Another approach to targeted therapy involves monoclonal antibodies coupled to therapeutic radioisotopes. An example of the use of this approach in melanoma has been reported by Larson *et al.* (12).

Since 1981, we have directed our efforts toward the development of immunotoxins consisting of monoclonal antibodies coupled to ribosomal inhibiting proteins, such as ricin A chain, as therapeutic modalities. These ribosomal inhibiting proteins are enzymes that inhibit protein synthesis by ribosomal inactivation, thereby causing cell death (13). Internalization of the A chain, which is necessary for cytotoxic action, occurs following the binding of the immunotoxin to the cell surface via the monoclonal antibody. There is evidence that ribosomal inhibiting proteins are so potent that one molecule entering the cytosol is sufficient to cause cell death (14).

In a radioimmunoimaging study, conducted under a United States FDA[3] Investigational New Drug exemption notice, using our monoclonal antimelanoma antibody labeled with indium-111, we showed that the antibody localizes in melanomas (15). This same antibody was coupled with ricin A chain to create an immunotoxin for therapy in which the antibody would bind to tumor cells and the A chain would kill the cells to which the antibody bound. We report herein the results of our trial of this monoclonal antimelanoma antibody-ricin A chain immunotoxin (XOMAZYME-MEL).

MATERIALS AND METHODS

Patients. The patients eligible for this study had Stage III metastatic malignant melanoma (disease spread beyond regional lymph nodes). The first eight patients were required to have failed chemotherapy with the last dose having been administered more than 4 weeks before immunotoxin therapy. After we gained clinical experience in immunotoxin administration with the first eight patients, we eliminated the requirement that patients must have received prior chemotherapy. Other criteria for entry into the study included that the patients have good performance status with an expected survival time of greater than

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[3] The abbreviations used are: FDA, Food and Drug Administration; EKG, electrocardiogram; i.d., intradermal.

3 months, a WBC ≥4,000 cells/cu mm, platelet count ≥100,000/mm³, good renal and liver function, and no severe systemic disease aside from melanoma. Signed, informed consent was obtained from all patients prior to entry into the study.

Monoclonal Antibody. For the generation of the hybridoma, the hybridization, cloning, and recloning were performed according to the conventional procedure described by Kohler and Milstein (2) with minor modifications (16). BALB/c mice were immunized with cultured human melanoma cells. The spleen cells were harvested and fused with the 8-azaguanine-resistant murine myeloma line P3-X63-Ag8 in the presence of polyethylene glycol. The cells were cultured overnight, resuspended in medium containing hypoxanthine, aminopterin, and thymidine. They were then cloned. Hybridomas secreting antibodies with the appropriate specificity were subcloned twice by limiting dilution using BALB/c splenocytes as feeder cells.

The monoclonal antibody used in the preparation of the immunotoxin was produced from murine ascites and was purified by XOMA Corporation (Berkeley, CA) using a staphylococcal Protein A column with elution at pH 3.5. It is an IgG2a antibody and reacts with melanoma-associated antigens having molecular weights of 220,000 and over 500,000. The hybridoma and the monoclonal antibody have been fully characterized in accordance with guidelines proposed by the FDA in the document entitled "Points to Consider in the Production of Monoclonal Antibody Products for Human Use." On frozen sections, the antibody shows minimal reactivity with normal tissues except for vascular endothelium, in which the reactivity appears to be cytoplasmic. The antibody also cross-reacts with nevus cells. The hybridoma contains xenotropic and ecotropic viruses; however, the purified antibody contains neither these viruses nor parental hybridoma DNA or RNA (17). Both the hybridoma and the purified antibody are free of other murine viral contaminants as determined by the mouse antibody production test (a test in which contamination with 12 murine viruses are evaluated by injection of the test article into mice and determination of antibody production to the viruses of interest).

XOMAZYME-MEL Immunotoxin. The immunotoxin, consisting of the murine monoclonal antimelanoma antibody conjugated to ricin A chain, is produced by XOMA Corporation. The conjugation technique has been described in detail elsewhere (18). The ricin A chain is purified by affinity chromatography using an anti-ricin B chain column. Briefly, the antibody is activated with *N*-succinimidyl-3-(2-pyridyldithio)propionate followed by addition of affinity purified ricin A chain which has been reduced with dithiothreitol. The immunotoxin is then purified by gel chromatography. It is provided in a sterile, pyrogen-free formulation at a concentration of 1.0 mg/ml in 0.9% phosphate-buffered saline solution, pH 7.0. Preclinical testing demonstrated binding specificity of the immunotoxin similar to that of the unmodified antibody and cytotoxic antimelanoma activity both *in vitro* and *in vivo*. Associated toxicology studies indicated safety of the drug.

Protocol. This study was conducted under a United States FDA Investigational New Drug exemption notice. Signed, informed consent was obtained from each patient. The patients' active participation in the study lasted for approximately 2 months, including an initial hospitalization of 7 to 12 days. Immediately after admission and prior to beginning therapy, each patient was skin tested by i.d. injection of 100 μg of the unmodified antibody is 0.1 ml phosphate-buffered saline, and the test site was observed during a 30-min interval for signs of immediate reactivity. All of the patients had negative skin tests.

Patients received the monoclonal antimelanoma antibody ricin A chain immunotoxin in doses ranging from 0.01 mg/kg/day for 5 days to 1 mg/kg/day for 4 days (Table 1). Two patients received a dose of 0.2 mg/kg/day for 10 days. The total dose administered during this study ranged from 3.2 to 300 mg. The immunotoxin was diluted to approximately 200 ml in phosphate-buffered saline and was administered by i.v. infusion over a period of 0.5 to 1.5 h.

The patients were evaluated serially by physical examination, ophthalmological examination, and laboratory tests including hematology panel, chemistry panel, urinalysis, creatinine clearance, prothrombin time, partial thromboplastin time, stool guaiac, complement levels, follicle stimulating hormone, estrogen, testosterone, chest X-ray, computerized tomography of the head, chest, and abdomen, EKG, and other laboratory tests as indicated. The IgG immune response to murine immunoglobulin and ricin A chain was also determined serially.

Immune Response. The antibody response to the immunotoxin components was measured in an enzyme immunoassay. Various dilutions of patients' sera were added to microtiter plates containing absorbed ricin A chain or the murine monoclonal antimelanoma antibody. Goat antihuman IgG antibody conjugated to alkaline phosphatase (Zymed Laboratories, South San Francisco, CA) was added followed *p*-nitrophenylphosphate (Sigma Laboratories, St. Louis, MO). Titration curves were generated for each patient, and the immune response was expressed as the ratio of the titration end point dilution of the serum sample showing maximum response to the titration end point dilution of the pretreatment serum sample.

Immunoperoxidase Studies. Biopsy specimens of tumors were obtained from study patients, and frozen sections were prepared for evaluation by two immunoperoxidase techniques, the avidin-biotin system and the indirect conjugate peroxidase method (19). Sections were stained for the presence of the immunoglobulin component of the immunotoxin by incubation with biotinylated horse anti-murine immunoglobulin antibody (Vector Laboratories, Burlingame, CA), followed by the addition of avidin-biotin complex (Vector). Parallel sections were stained for the ricin A chain component of the immunotoxin using a goat anti-ricin A chain antiserum, followed by a biotinylated rabbit anti-goat immunoglobulin reagent (Vector) and avidin-biotin complex. Additional studies were performed using an indirect conjugate peroxidase method with similar results. Sites of localization of the immunoperoxidase were revealed by the use of 3-amino-9-ethylcarbazole as the chromogen, giving a red color that is distinguishable from melanin pigment. Tissues were examined independently by two observers.

RESULTS

Twenty-two patients were evaluated in this study (Table 1). There were 13 males and 9 females with ages ranging from 28 to 69 years. All had Stage III melanoma; 13 had failed prior chemotherapy and 9 had not received chemotherapy.

Side effects associated with immunotoxin administration are summarized in Table 2. The severity of the side effects was generally related to the dose of immunotoxin administered. In doses up to and including 0.5 mg/kg/day for 5 days, these side effects were transient and reversible in all patients evaluated. The major dose-limiting side effect of this immunotoxin was a

Table 1 *Patient population included in the trial of XOMAZYME-MEL monoclonal antimelanoma antibody ricin A chain immunotoxin*

Patient	Age (years)	Sex	Prestudy lactic dehydrogenase	Daily dose (mg/kg)	Doses received	Total dose (mg)
1	31	F	N[a]	0.01	5	3.2
2	56	F	N	0.05	5	16
3	39	F	N	0.2	5	68
4	36	M	E	0.2	5	67
5	28	M	N	0.5	5	260
6	51	M	E	0.5	5	180
7	52	M	E	1.0	4	300
8	35	F	E	1.0	4	200
9	53	F	N	0.75	5	298
10	44	M	E	0.2	10	135
11	33	M	E	0.5	5	190
12	38	F	E	0.75	5	195
13	45	F	E	0.2	10	106
14	41	M	E	0.5	5	250
15	55	M	N	0.75	4	265
16	36	F	E	0.75	2	80
17	54	M	N	0.5	5	300
18	41	M	N	0.5	5	183
19	62	M	N	0.5	5	235
20	58	M	N	0.5	5	190
21	68	F	N	0.5	5	170
22	57	M	N	0.5	5	200

[a] N, normal; E, elevated.

Table 2 *Clinical and laboratory findings in patients undergoing immunotoxin therapy*

Observations	No. of patients
Clinical	
Malaise/fatigue	15
Fever	14
Tachycardia	14
Decreased appetite	12
Nausea	6
Weight gain (>5%)	6
Myalgia	5
Flush	2
Death	1[a]
Pruritus	1
Laboratory	
Albumin decrease (>10%)	20
Total serum protein decrease	20
Low voltage on EKG	16
Fibrinogen increase	12/13[b]
Erythrocyte sedimentation rate increase	8/11
Leukocytosis	8
Calcium decrease	7
Serum glutamic oxaloacetate transaminase increase[c]	6[d]
Lactic dehydrogenase increase[c]	2[d]
Thrombocytopenia (<50,000 platelets/cu mm)	2[d]
PTT prolongation	2[d]
C reactive protein increase	2/2
Eosinophilia	1[d]
Creatinine increase	1[d]
Hemoconcentration	1
Metabolic acidosis	1

[a] Relationship to immunotoxin therapy not established (see text).
[b] Denominator represents number of patients evaluated if observations were not made in all 22 patients.
[c] Increase to two or more times the baseline value.
[d] Some of the abnormal values were attributed to disease progression or other causes (see text).

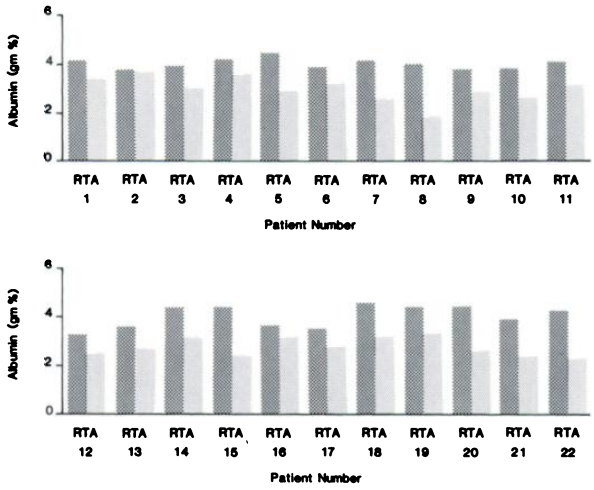

Fig. 1. Results of albumin levels in 22 patients receiving immunotoxin. Individual prestudy levels and nadir of albumin levels are shown for each patient. Dosages are indicated in Table 1. *Lighter bars*, prestudy albumin level; *darker bars*, modes of albumin level; *gm %*, g/100 ml.

fall in serum albumin with a concomitant decrease in serum total protein values. Weight gain and fluid shifts were noted which resulted in edema.

The fall in serum albumin was observed in all patients receiving a dose of at least 0.2 mg/kg/day of immunotoxin (Fig. 1). This decrease usually occurred after the second dose of immunotoxin. Serum albumin levels in 10 patients given 0.5 mg/kg/day for five days are illustrated in Fig. 2. For those patients, the mean serum albumin level was 4.2 ± 0.1 (SE) g/100 ml before treatment and fell to a nadir of 2.9 ± 0.1 g/100

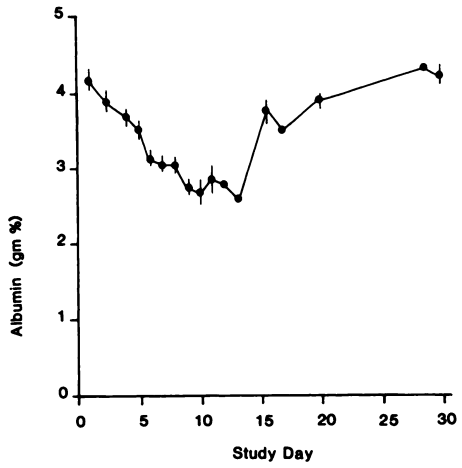

Fig. 2. Results of serial serum albumin levels in 10 patients receiving 5 infusions of immunotoxin at a dose of 0.5 mg/kg/day within an 8-day period. *Numbers*, mean ± SE (*bars*); *gm %*, g/100 ml.

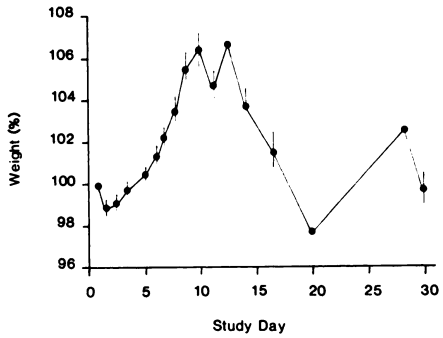

Fig. 3. Results of serial body weight determinations in 10 patients receiving 5 infusions of immunotoxin at a dose of 0.5 mg/kg/day within an 8-day period. *Numbers*, mean ± SE (*bars*).

ml. There was no associated proteinuria. Serum albumin levels stabilized and began to return to normal within 48 h of the last treatment with immunotoxin.

All patients receiving a dose of at least 0.2 mg/kg/day of immunotoxin gained weight during the course of hospitalization for immunotoxin treatment. The weight change for the 10 patients who received 5 infusions of immunotoxin at a dose of 0.5 mg/kg is illustrated in Fig. 3. For this group, the weight gain was 9.1 ± 1.3 lb (range, 1 to 17.4 lb). Associated with this were clinical signs of edema in some patients. There were no signs of pulmonary edema in any patient. The patients' weights decreased to baseline levels as the serum albumin returned to pretherapy levels.

Along with the clinical evidence of fluid retention, most patients receiving a dose of at least 0.5 mg/kg/day of immunotoxin exhibited signs of mild hypovolemia as manifested by tachycardia and, in some cases, a modest fall in orthostatic blood pressure. These signs and symptoms were not severe and responded to conservative management. In some patients, daily doses of immunotoxin were interrupted for 1 to 2 days to allow for partial reversal of these transient effects before continuing therapy. I.v. hydration was administered to some patients.

Many of the patients manifested fevers several hours after infusion of immunotoxin, and they generally ranged between 38 and 39°C. In addition, most patients experienced malaise, fatigue, and decrease in appetite during the course of hospital-

ization. Some patients had associated myalgia. These side effects were self-limiting and well tolerated.

A transient change to decreased voltage on EKG without other ST-T wave changes or clinical symptoms was noted. Sinus tachycardia was also clinically observed in most patients. Serial echocardiograms and creatine kinase fraction 2 levels [CK_2(MB)] were obtained in four patients who showed low voltage on EKG. There was no evidence of significant pericardial effusions or changes in ventricular function. Levels of creatine phosphokinase muscle-brain isozyme did not increase.

A review of the hematological evaluation showed no evidence of bone marrow suppression. There was a moderate leukocytosis with an associated decrease in the percentage of lymphocytes in some patients. Platelet counts generally did not fall below normal values. In 4 patients, platelet counts increased modestly during the course of therapy. Two patients had prolongation of partial thromboplastin time values to more than 110 s and thrombocytopenia with platelet counts of less than 50,000 platelets/mm^3. One of these patients had rapidly progressive metastatic melanoma with secondary disseminated intravascular coagulation and a heparin-induced thrombocytopenia. This coagulopathy was associated with guaiac-positive stools. The etiology of the second patient's abnormal coagulation parameters is unknown and was not associated with any bleeding diathesis. In addition, there was a fall in platelets from a pretreatment value of 120,000 mm^3 to a nadir of 76,000 in one patient (no. 16) that was attributed to a recent course of chemotherapy with lomustine.

Twelve of 13 patients evaluated had an increase in serum fibrinogen during immunotoxin therapy. There was also noted an increase in erythrocyte sedimentation rate and C reactive protein in some patients. Some patients also demonstrated mild transient elevations in lactic dehydrogenase and serum glutamic oxaloacetate transaminase levels. In only two patients was there an increase to five or more times the baseline value. One of these patients (no. 8) had rapidly progressive disease, and in the other (no. 15), the increase was a preterminal event. Seven patients demonstrated decreases in serum calcium which correlated with the decrease in serum albumin. This decrease probably reflects a fall in available albumin for calcium binding. There was no clinical evidence of hypocalcemia, and the calcium levels returned to normal as albumin levels returned to baseline values.

In one patient (no. 17), with known preexisting gout and associated nephropathy, there was a transient increase of creatinine from 1.3 to 1.9 mg/dl associated with increased activity of his gout during the course of hospitalization for immunotoxin therapy. These changes were attributed to a combination of the preexisting nephropathy with mild prerenal azotemia and medication with indomethacin.

Three patients experienced possible allergic reactions. Two of these had received prior murine antibody as a part of the previously mentioned radioimmunoimaging study, and one experienced the reaction after a 10-day course of infusions. In two patients, the reactions occurred during the infusion and consisted of facial flush and slight nausea. There was associated pruritus in one of these patients. In another patient, the reaction resembled an atypical serum sickness with eosinophilia. All reactions were mild and resolved spontaneously without medication except that one patient was given one p.o. dose of diphenhydramine for symptomatic relief of pruritus.

Three of 22 patients in this study expired within 2 months of receiving immunotoxin therapy. Two patients had rapidly progressive metastatic melanoma and died as a result of their widespread disease. The third patient (no. 15), who had a prior history of coronary artery disease with apparent unstable angina, demonstrated hypotension, hemoconcentration, and a metabolic acidosis within 12 h after the fourth dose of immunotoxin (0.75 mg/kg/day). The patient initially responded to i.v. hydration, but he developed atrial fibrillation and had a cardiopulmonary arrest within 36 h of his last dose of immunotoxin. Autopsy findings confirmed his past history of coronary artery disease but did not clarify the cause of death. The immediate cause of death was thought to be due to an arrhythmia. The relationship between immunotoxin administration and this patient's death is unknown.

Pharmacological studies were performed and will be the subject of a subsequent manuscript. Immunological analysis indicated that almost all patients mounted an immune response to both the murine immunoglobulin and ricin A chain components of the immunotoxin following completion of the therapeutic course (Fig. 4). Results of these studies will be presented in detail elsewhere. Encouraging clinical results were observed in this study (Table 3). One patient had a complete response with disappearance of a pulmonary metastasis. The response is ongoing at 13 months. In addition, four patients had a mixed response which is defined as a 50% or greater reduction in area of one or more metastases while concurrently one or more lesions increased in size or a new lesion appeared after treatment. Although these do not meet the standard oncological definition of an objective response, they are noteworthy in view

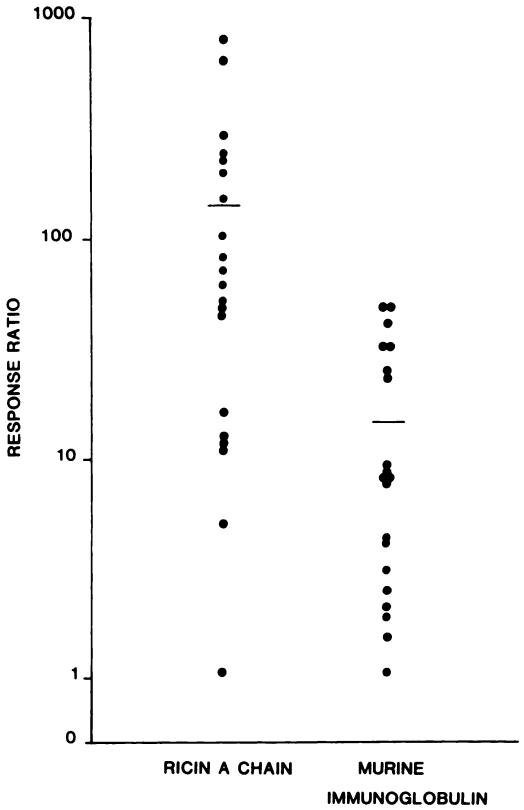

Fig. 4. IgG antibody response to immunotoxin components. For each patient, the immune response is expressed as the ratio of the titration end point dilution of the serum sample showing maximum response to the titration end point dilution of the pretreatment serum sample.

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