Ganglioside G_{D2} Specific Monoclonal Antibody 3F8: A Phase I Study in Patients With Neuroblastoma and Malignant Melanoma

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The murine IgG_3 monoclonal antibody (MoAb) 3F8, specific for the ganglioside G_{D2}, activates human complement, is active in antibody-dependent cell-mediated cytotoxicity (ADCC), and can target specifically to human neuroblastoma in patients with metastatic disease. In a phase I study, 3F8 was administered intravenously (IV) to 17 patients with metastatic G_{D2} positive neuroblastoma or malignant melanoma at doses of 5, 20, 50, and 100 mg/m^2. Serum 3F8 levels achieved were proportional to the dose of 3F8 infused. However, serum antimouse antibody levels did not increase with the amount of 3F8 administered. Toxicities included pain, hypertension, urticaria, and complement depletion. All acute side effects were controllable with symptomatic therapy. No long-term side effects were detected in patients observed for more than 14 months. None of the 17 patients received any antitumor therapy postantibody treatment. Antitumor responses occurred in seven of 17 patients. These ranged from complete clinical remissions to mixed responses. The murine monoclonal antibody (MoAb) 3F8 has clinical utility for the diagnosis and therapy of neuroblastoma and melanoma.

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MONOCLONAL ANTIBODIES (MoAb) to human tumors have both diagnostic and therapeutic potentials.[1] In the absence of radioisotope, drug, or toxin immunoconjugates, MoAb can have therapeutic effects in patients with various malignancies, such as chronic lymphocytic leukemia,[2] gastrointestinal tumors,[3] and malignant melanoma.[4,5] The mechanism of action of such MoAb may be related to their complement activation properties,[4,5] and antibody-dependent cell-mediated tumor cytotoxicity (ADCC).[6,7]

We have described a murine MoAb 3F8 specific for the disialoganglioside G_{D2}.[8,9] Immunofluorescence and immunoperoxidase staining studies have shown that antigen G_{D2} has a restricted distribution in normal human tissues. However, it reacts strongly with human neuroblastoma, melanoma, sarcoma, and small-cell lung cancer, as well as brain tumors. This IgG_3 antibody is capable of activating human complement in tumor cytotoxicity[10] and ADCC in vitro. It has also been shown that 3F8 inhibits tumor cell attachment to extracellular matrix proteins.[11] When radiolabeled, the antibody can target specifically to G_{D2} positive tumors, such as human neuroblastoma, in patients with metastatic disease.[12] We now report a phase I clinical study using antibody 3F8 in 17 patients with metastatic melanoma and neuroblastoma. Major antitumor responses were noted in patients with widespread disease.

METHODS

Patients

Eight patients with neuroblastoma and nine patients with malignant melanoma were entered into the study. All had recurrent or progressive metastatic disease, and most of them have failed intensive chemotherapy and radiation therapy. The presence of G_{D2} antigen in patients' tumors was tested by immunoperoxidase and immunofluorescence staining[8] on frozen sections before treatment. All patients had measurable disease, a performance status (Karnofsky scale) of at least 60%, and had been off anticancer therapy for at least 4 weeks. No concurrent anticancer therapy (except palliative radiation therapy in patients no. 16 and 17) was administered during evaluation. Patients were followed for tumor response for at least 6 weeks after initiation of therapy. This phase I study was approved by the Institutional Review Board of University Hospitals of Cleveland. Written informed consent was obtained from every patient or parents in cases of minors.

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3F8 Preparation

The 3F8 was prepared from ascites of Balb/c mice and purified by ammonium sulfate precipitation and chromatography over protein A-sepharose.[7] Each batch was tested for antibody activity, purity by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis, and assayed for the absence of nucleic acids, murine viruses, bacteria, fungi, and mycoplasma. Preparations passed the safety testing in mice and guinea pigs, and pyrogenicity testing in rabbits.

Diagnostic Imaging With Iodine 131 Labeled 3F8

The 3F8 was radiolabeled with iodine 131 using the chloramine T method and then used for diagnostic imaging.[12,13] Patients' thyroid glands were protected by the administration of saturated solution of potassium iodide orally twice a day for 14 days. Between 2.5 to 5 mCi of radioactive antibody, carried on 250 to 500 μg of 3F8, was injected intravenously (IV). The patients were scanned using a Searle LFOV camera (Searle Radio-graphics, Des Plaines, IL) equipped with a high energy collimator attachment, and the information was simultaneously acquired on film and on a Medical Data Systems computer (MDS, Ann Arbor, MI). The camera was set for the 364 gamma peak, with a 20% window.

3F8 Antibody Administration

All patients were shown to be nonreactive by skin testing with 50 μg of 3F8 before antibody treatment. The 3F8 was administered by IV infusion in 5% human serum albumin/0.9% saline, at the rate of 1 to 10 mg/h over eight hours per day from two to four days. Except for patient no. 7, all patients were administered antibody 3F8 infusion less than seven days after the imaging dose. The following escalating dosages (5, 20, 50, and 100 mg/m^2) were chosen in view of the published experience with R_{24}, an IgG_3 murine MoAb specific for the ganglioside G_{D3}.[4] The study was closed at the 100 mg/m^2 dose, because all six patients receiving this dose of 3F8 developed significant hypertension.

Serological Tests

Serum concentrations of 3F8 were measured by enzyme-linked immunosorbent assay (ELISA). Ninety-six well polyvinylchloride microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated with 1 μg/mL of purified 3F8, at 100 μL per well. Rabbit anti-mouse IgG_3 (Miles Scientific, Naperville, IL) diluted at 1:1,000 was incubated (1:1 v/v), with 1:10 dilutions of patients' serum samples and incubated for two hours at 37°C. The reaction mixture was transferred to precoated wells, incubated for 60 minutes, and washed with cold phosphate buffered saline (PBS) with 0.2% bovine serum albumin (BSA) (Sigma, St Louis). Wells were incubated with peroxidase conjugated goat anti-rabbit IgG (Tago, Burlingame, CA) for 60 minutes. Plates were then washed with PBS-BSA, and the substrate o-phenylenediamine (Sigma), at 0.5 mg/mL in 0.1 citrate phosphate buffer pH 5, was added (150 μL per well). Color reaction was stopped with 30 μL of 5N H_2SO_4, and read with a Dynatech ELISA plate reader at 492 nm. The standard used was 3F8 diluted in normal human serum.

Human IgG antibody against 3F8 was measured by ELISA. Polyvinyl chloride plates were coated with 2 μg/mL of purified 3F8 (100 μL per well). Serum was diluted to 1:100 and reacted with the wells for 60 minutes at 37°C. Plates were washed with PBS-BSA and then reacted with a 1:3,000 dilution of peroxidase conjugated affinity purified goat anti-human IgG (Tago) at 37°C for 60 minutes. The plates were washed and the substrate o-phenylenediamine added as described.

Indirect immunofluorescence and immunoperoxidase procedures were carried out as described.[8] 3F8 and a control antibody against sheep red cell were used at 20 μg/mL.

Evaluation of Tumor Responses

Patients were staged by physical examinations; complete blood counts; serum tests of liver function; computerized tomography (CT) scans of head, chest, abdomen, and pelvis; bone scans; bone marrow aspirates; and biopsies. Twenty-four hour urine catecholamines also were collected in patients with neuroblastoma. Patients were followed at weekly intervals for 12 weeks, then monthly for a total of 12 months. Complete response (CR) is defined as the disappearance of all signs and symptoms, and biochemical and x-ray evidence of tumor. In the case of cutaneous, subcutaneous, or bone marrow metastases, tissue biopsy of at least one known site of tumor was repeated before judging a response complete. Partial response (PR) is defined as a reduction of all measurable tumors by at least 50% of the sum of the product of the two greatest diameters. Mixed response (MR) is a reduction in size of some measurable tumors (but less than that for PR). Stable disease (SD) is no objective change of all measurable tumors. Progressive disease (PD) is the absence of response of any lesions and the appearance of new lesions or increase in size of any measurable tumor by at least 25% of the product of two greatest diameters. All pathological materials, including aspirates and tissue sections before and after therapy, were reviewed.

RESULTS

Patient Characteristics

Table 1 summarizes the clinical features of the 17 patients included in the study. Eight patients had neuroblastoma and nine patients had malignant melanoma. Two patients received 5 mg/m^2, five received 20 mg/m^2, four received 50 mg/m^2, and six received 100 mg/m^2 of 3F8. The age ranged from 17 months to 20 years among the neuroblastoma patients, and 22 to 56 years among the melanoma patients. All except four melanoma patients had failed prior chemotherapy, radiation therapy, or bone marrow transplantation. Patients no. 1, 3, 8, and 17 were on corticosteroids when they were enrolled in this clinical trial. All 17 patients had progressive metastatic disease at the time they entered the study: ten had bone, nine had lymph nodes, and seven had bone marrow involvement.

Table 1. Clinical Features and Tumor Responses of Patients Treated With 3F8

Patient No.	Age/Sex	Tumor Type Primary Site	Prior Therapy	3F8 Dose (mg/m^2)	Metastasis		
					Sites of Disease	Response	Duration (wk)
1	12/F	NB Adrenal	VCR/CPM/DTIC BMT (L-PAM + TBI), focal XRT	5	Bone marrow	CR	63
2	38/F	Mel Trunk	CPM/DTIC/BCNU	5	Skin, lymph nodes	SD	10
3	5/F	NB Adrenal	VCR/CPM/DTIC ADRIA/CDDP, BMT (L-PAM + TBI), focal XRT	20	Bone, bone marrow	CR	28
4	6/F	NB Adrenal	VCR/CPM/DTIC VP-16/CDDP/L-PAM, aBMT (Thiotepa) × 2, focal XRT	20	Bone, abdomen	PD	12
5	22/F	Mel Trunk	None	20	Lymph nodes	PR	22
6	43/M	Mel Mediastinum	VCR/DTIC Tamoxifen, aBMT (L-PAM + BCNU) × 2, focal XRT	20	Skin, lymph nodes, muscle, lung, adrenal	MR	16
7	56/M	Mel	None	20	Lymph nodes, mediastinum	PD	
8	1.5/M	NB Adrenal	CPM/ADRIA VP-16/CDDP; focal XRT	50	Skin	SD	24
9	2/M	NB Adrenal	VCR/CPM/DTIC aBMT (VAMP + TBI)	50	Bone, bone marrow	SD	4
10	30/F	Mel Leg	Focal XRT	50	Bone, liver	PR	56+
11	43/M	Mel	DTIC/BCG	50	Skin, lymph nodes	SD	6
12	6/F	NB Adrenal	VCR/CPM/DTIC L-PAM, aBMT (VAMP + TBI)	100	Bone	PD	
13	12/F	NB Mediastinum	VCR/CPM/DTIC BMT (L-PAM/CPM + TBI)	100	Liver, ascites, bone, bone marrow	PD	
14	21/M	NB Olfactory Nerve	VCR/CPM/VP-16	100	Skin, lymph nodes, soft tissues	PD	
15	28/M	Mel Trunk	None	100	Skin, lymph nodes, bone, bone marrow	MR	6
16	35/F	Mel Leg	None	100	Lymph nodes, bone, bone marrow	PD	
17	51/M	Mel Foot	Focal XRT	100	Skin, lymph nodes, bone, bone marrow, brain	PD	

Abbreviations: NB, neuroblastoma; Mel, melanoma; VCR, vincristine; CPM, cyclophosphamide; BMT, allogeneic bone marrow transplantation; aBMT, autologous bone marrow transplantation; L-PAM, melphalan; TBI, total body irradiation; XRT, radiotherapy; CDDP, cisplatin; ADRIA, Adriamycin (Adria Laboratories, Columbus, OH); VAMP, combination of VM-26 or VP-16, Adriamycin, melphalan, and cisplatin.

In Vitro and In Vivo 3F8 Binding to Tumor

In order to determine the presence of G_{D2} in tumors before treatment, the binding of 3F8 to tumors was studied in vitro (immunostaining of biopsied tumor tissues), as well as in vivo (radioimaging with ^{131}I-3F8). The results are summarized in Table 2. All 17 patients showed binding by immunostaining and/or by radioimaging. Among the neuroblastoma patients, five had strong 3F8 binding by in vitro staining (3 to 4+ on a scale of 4, and 100% cells stained) as well as by in vivo imaging. In two patients, radioimag-

Table 2. Tumor Binding of Antibody 3F8

Diagnosis	No. of Patients	In Vitro Immunostaining	In Vivo Radioimaging
Neuroblastoma	5	+	+
	2	+	ND
	1	ND	+
Melanoma	4	+	+
	3	+	−
	1	ND	+
	1	+	ND

Abbreviation: ND, not done.

ing was not done (ND). In one patient, radioimaging was positive although no tumor was available for in vitro studies. Among the nine melanoma patients, four were positive both in vitro and by in vivo radioimaging. In melanomas, immunostaining showed heterogeneity of intensity (2 to 4+) and percentage of positive cells (10% to 100%). Three patients showed no definite uptake of radioactivity in tumor sites, although their tumor reacted with 3F8 in vitro. One patient had no tissue available for testing in vitro, but showed uptake in the imaging study. One patient was positive by immunostaining, but radioimaging was not performed. Figure 1 shows a representative patient (no. 3) with neuroblastoma two days after IV injection with ^{131}I-labeled 3F8. The panels (1C, 1D, 1E) represent antibody scans of several anatomic regions. Except for the areas of free iodine in the stomach and in the urinary bladder, the scans demonstrate focal lesions in the cranium, spine, pelvis, and upper and lower extremities. Four patients with skin tumors had tissue available to study mouse IgG tumor localization within seven days after the antibody was given. These patients received ≥ 50 mg/m^2, and they all showed deposition of mouse IgG in the tumor (data not shown).

Toxicity

The major toxicities encountered during the antibody infusion included severe pain, hypertension, and focal urticaria. The pain pattern typically involved the abdomen, lower back, and sometimes the chest, and tended to spread peripherally to the ankles and the feet. It usually started within one hour of antibody infusion and continued until the infusion was stopped. All patients studied developed pain irrespective of antibody dose and all patients required treatment with analgesics. Two patients who received 20 mg/m^2 and 50 mg/m^2 of 3F8, respectively, described a slight decrease in sensitivity to heat and cold for 4 weeks after the antibody treatment. The former patient also described mild diffuse arthralgia that slowly disappeared over 3 months. There were no long-term objective neurological deficits (motor or sensory) in the 17 patients (eight of them observed for at least 6 months). Patients no. 6 and 11 developed painful swelling around the skin nodules within 2 weeks after antibody infusion. Significant elevation of diastolic BP was seen in most of the patients, particularly those who received 100 mg/m^2 of antibody. An increase of more than 40% of baseline arterial pressure was recorded in nine patients (Table 3). Hypertension was temporally related to the antibody infusion and quickly resolved after it was discontinued. However, two neuroblastoma patients had persistent hypertension requiring oral antihypertensives for 2 weeks. Urticarial rashes were always focal, independent of dose level, and easily reversible with antihistamines. Febrile reactions were more common in patients receiving 100 mg/m^2 of 3F8. A decrease in serum complement activity (CH50) was seen in patients receiving ≥ 20 mg/m^2. However, there was no correlation between antibody dose and the degree of complement depletion. Four patients receiving glucocorticoids (8 to 120 mg/m^2 prednisone) showed a mean decrease in the CH50 of 12% $\pm$ 14%, while the other 13 patients showed a mean decrease of 26% $\pm$ 15%. C3 and C4 showed similar changes. Patients receiving ≥ 100 mg/m^2 antibody developed mild nausea and diarrhea within the 2 weeks after the antibody was administered. Except for one patient who developed a transient increase in serum creatinine (to 1.9 mg/dL) after a hypotensive episode from IV morphine, none of the patients had any signs of renal dysfunction. No hematopoietic, hepatic, or pulmonary toxicity was observed, and no changes were noted in vision or skin pigmentation over a period of up to 14 months of observation. No anaphylactic reactions occurred.

Serology

The peak serum 3F8 level after antibody infusion was measured and the arithmetic means calculated at each dose level (see Table 4). As the

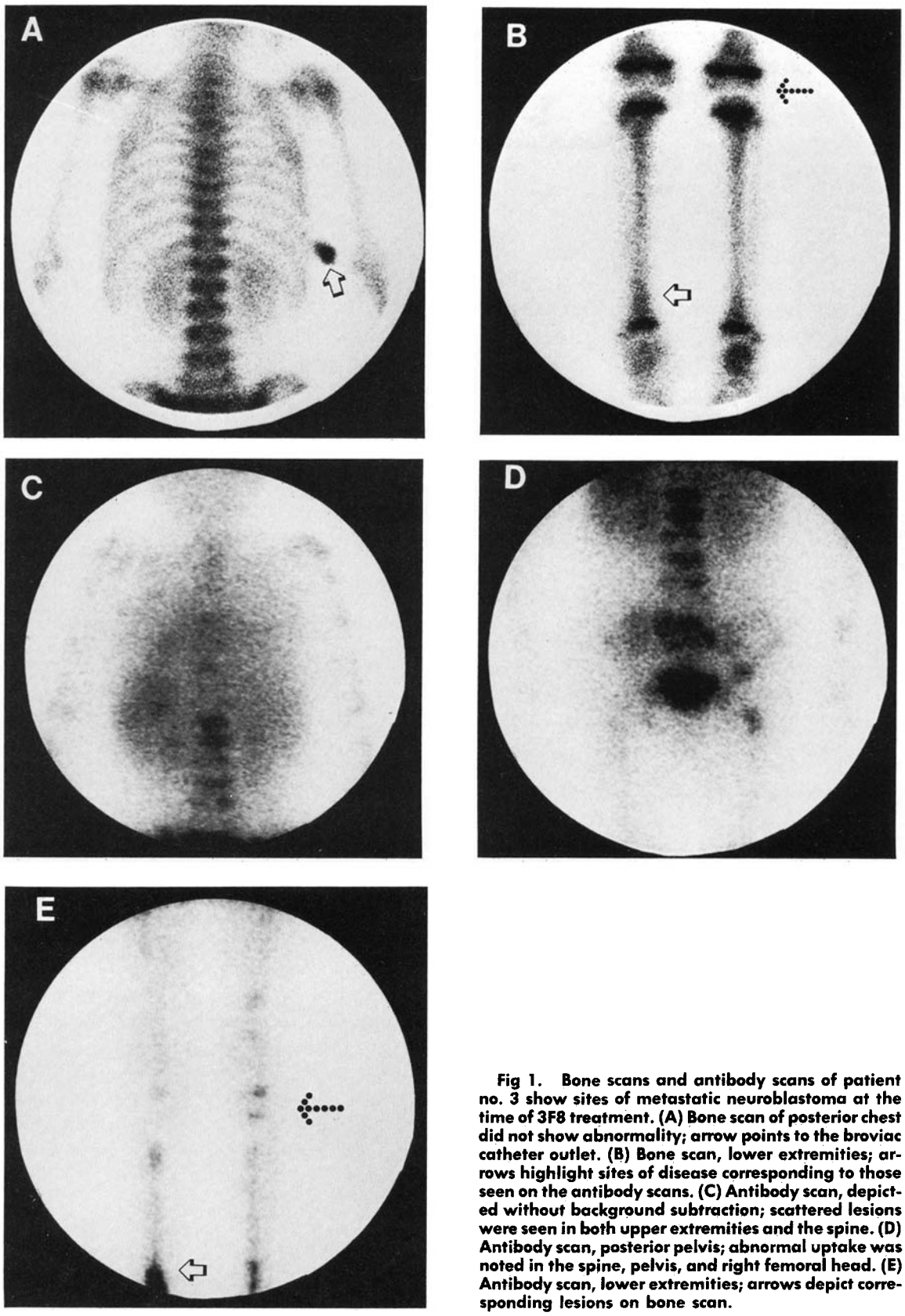

Fig 1. Bone scans and antibody scans of patient no. 3 show sites of metastatic neuroblastoma at the time of 3F8 treatment. (A) Bone scan of posterior chest did not show abnormality; arrow points to the broviac catheter outlet. (B) Bone scan, lower extremities; arrows highlight sites of disease corresponding to those seen on the antibody scans. (C) Antibody scan, depicted without background subtraction; scattered lesions were seen in both upper extremities and the spine. (D) Antibody scan, posterior pelvis; abnormal uptake was noted in the spine, pelvis, and right femoral head. (E) Antibody scan, lower extremities; arrows depict corresponding lesions on bone scan.

Table 3. Toxicities

Group No.	Dose (mg/m^2)	Pain	Diastolic BP Elevation >40%	Urticaria	Oral Temperature >38.5°C	Mean Decrease in CH50 (%)
1	5	Severe	0/2	1/2	0/2	6
2	20	Severe	2/5	4/5	0/5	25
3	50	Severe	1/4	4/4	1/4	28
4	100	Severe	6/6	5/6	3/6	20

MoAb dose was increased from 5 to 100 mg/m^2, the mean serum 3F8 level increased. Human anti-mouse antibody titer was low to undetectable (<92 arbitrary U/mL, which is the upper limit of unimmunized patients and volunteers) 12 days after the first antibody exposure. The peak response occurred usually within 30 days after infusion. The geometric means of the antibody titer in units per milliliter were not higher in patients receiving higher doses of 3F8. Patients with < 1,000 U/mL anti-mouse response had expected side effects and therapeutic benefits from repeated 3F8 infusions (data not shown). Three of five patients receiving 20 mg/m^2, three of four patients receiving 50 mg/m^2, and four of six receiving 100 mg/m^2 of 3F8 developed peak anti-mouse antibody levels of < 1,000 U/mL.

Antitumor Effects

Table 1 summarizes the tumor responses in patients treated with 3F8. Major tumor regression was observed in four patients (no. 1, 3, 5, and 10).

Patient no. 1 was a 12-year-old girl with stage IV neuroblastoma. She failed conventional chemotherapy and later allogeneic bone marrow transplantation (BMT). Her posttransplant marrow showed active disease by conventional histology and G_{D2} positive cells by immunofluorescent staining with 3F8. Because of rising urine catecholamines, progressive thrombocytopenia, and diffuse bone pain, she received focal radiation to both knees and 5 mg/m^2 of 3F8 IV. Four weeks after the treatment, her platelet count became normal and leg pains resolved. Eight weeks after treatment, the urine catecholamines became normal. She continued to have no evidence of tumor without further therapy. Forty-eight weeks after antibody treatment, her bone marrow continued to be free of neuroblastoma by histology, as well as by immunofluorescence. At 63 weeks, she died of interstitial pneumonitis. An autopsy was not performed.

Patient no. 3 is a 5-year-old girl with stage IV neuroblastoma. She failed conventional chemotherapy and later allogeneic BMT. Posttransplant, she continued to have active bone marrow disease (by biopsy and by immunofluorescence with antibody 3F8) and bony disease by bone scan and 3F8 antibody scan (Fig 1). She received a 20 mg/m^2 dose of 3F8. Four weeks after treatment, her platelet count became normal. Seven weeks after treatment, her marrow became free of disease (aspirate, biopsy, and immunofluorescence). Frequent marrow studies performed every 1 to 2 months showed continuous remission. Her urine catecholamines and blood counts also became normal. By 16 weeks after antibody treatment, all the previously abnormal bony lesions became normal. At 28 weeks, she showed

Table 4. Serology

Group No.	Dose (mg/m^2)	No. of Patients	Peak Serum 3F8 ($\mu g/mL$)	Peak Human Anti-Mouse Antibody Response Mean U/mL (range)
1	5	2	1.1 ± 0.6	4,647 (1,900-18,000)
2	20	5	3.7 ± 1.4*	722 (270-7,400)
3	50	4	14.9 ± 2.9	503 (50-46,000)
4	100	6	22.4 ± 7.6	1,149 (50-25,000)

*Patient no. 7 was sensitized by the imaging dose of 3F8 5 weeks before the phase I study; he had no detectable circulating mouse IgG_3 in the serum after antibody infusion and was not included in the analysis of peak serum 3F8 levels.

an isolated asymptomatic area of uptake on the right parietal skull on bone scan. At 34 weeks, her marrow continued to be free of disease with normal blood counts. Her total body CT and bone scan did not reveal other lesions. The isolated cranial bone lesion was excised and showed recurrent neuroblastoma that was G_{D2} positive. Since she had no significant circulating anti-mouse antibody, she was reimaged with the ^{131}I-radiolabeled 3F8. The antibody scans showed three focal areas of uptake in the cranium, while all the previous sites of disease have disappeared. At 45 weeks after antibody treatment the patient is alive with recurrent disease in cranium and surrounding soft tissues.

Patient no. 5 is a 21-year-old male with a malignant melanoma (stage II, Clark level III-IV) removed from his posterior thorax in December 1984. A left axillary node metastasis was removed in April 1985. After the development of extensive right axillary, paraesophageal, and pelvic nodal metastases, he received a 20 mg/m^2 dose of 3F8. Nine weeks after treatment, he had complete resolution of all paraesophageal and pelvic metastases (Fig 2). His right axillary node showed changes consistent with necrosis on CT scan. Twenty-two weeks after treatment, he developed new paraaortic and peripancreatic metastases in his abdomen. He was not retreated.

Patient no. 10 is a 30-year-old woman with melanoma removed from her right leg, which metastasized to her inguinal nodes in September 1982. She developed biopsy-proven metastasis to her distal right femur as well as liver metastasis on CT scan. She received 50 mg/m^2 of 3F8 IV and 3,600 rad to the right knee. Twelve weeks after treatment, her liver CT scan improved. At 36 weeks, her liver CT scan was normal. Her bone scan continued to improve. There were no new bone lesions and she was free of disease on repeat bone biopsy. She is fully ambulatory and continues in remission 56 weeks after treatment.

Two patients had mixed responses to 3F8. Patient no. 6 had metastatic melanoma, which did not regress after two autologous BMT. He developed new skin lesions and new metastatic disease to the rectus spinalis muscle and adrenal glands. He received 20 mg/m^2 of antibody. After 4 weeks, three of four skin nodules, the muscle, and adrenal metastases completely resolved by CT scan (Fig 3). Previously noted disease in the mediastinum and lung did not show any change. Patient no. 15 had metastatic melanoma to his bone, bone marrow, and lymph nodes. After 100 mg/m^2 of antibody, he had less than a PR of his right axillary node and his superficial skin nodules. Patient no. 4 was a 6-year-old girl with stage IV neuroblastoma. She failed two autologous BMTs and received 20 mg/m^2 of 3F8. Ten weeks after antibody treatment, her bone scan revealed resolution of bone lesions, but also appearance of new foci. Her abdominal tumor did

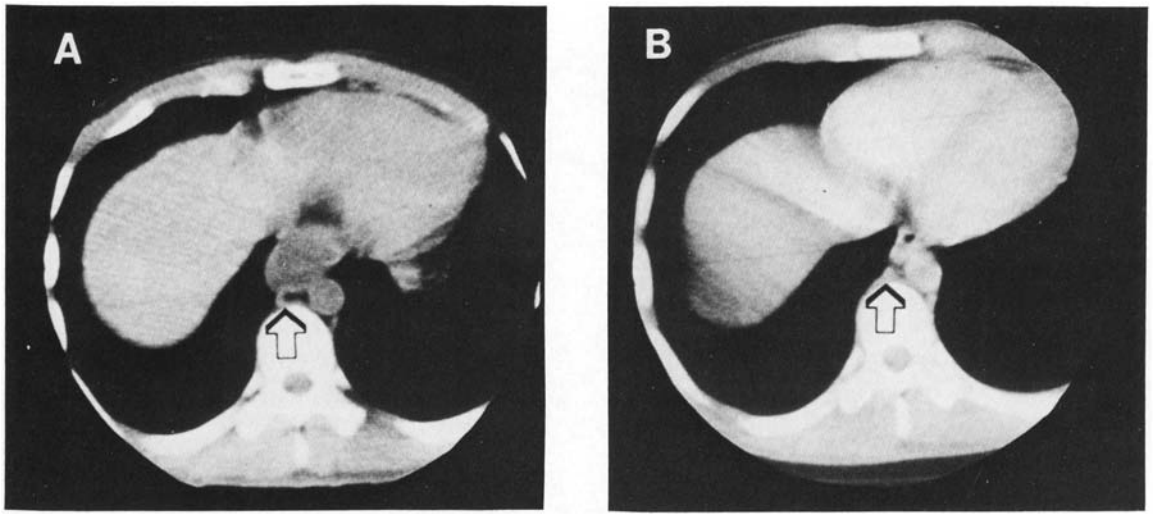

Fig 2. Chest CT scans of patient no. 5 show mediastinal metastases of malignant melanoma. (A) Before 3F8 treatment; arrows point to sites of disease. (B) After 3F8 treatment.

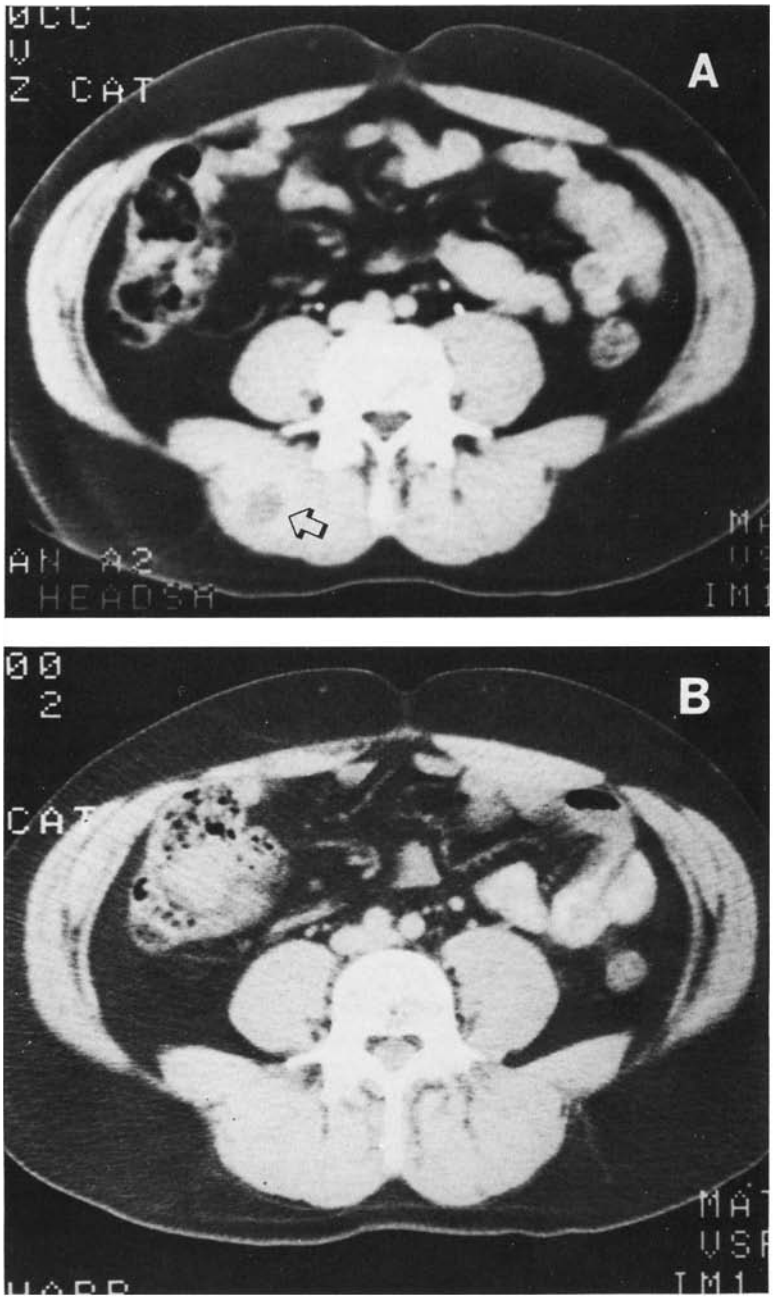

Fig 3. Abdominal CT scans of patient no. 6 show metastatic melanoma in the rectus spinalis muscle (arrow). (A) Before 3F8 treatment. (B) After 3F8 treatment.

not show any response. Because of the appearance of new foci on bone scan, she was evaluated as PD.

Follow-Up Studies of Bone Marrow and Biopsy Specimens

Six of the seven patients had bone marrow samples studied again after the antibody was administered. Percent tumor cells was calculated based on the total number of nucleated cells (tumors, and myeloid and erythroid precursors) seen on Wright and hematoxylin staining of the bone marrow aspirates and biopsies. Percent G_{D2} positive cells was also measured. Patients no. 1 and 3 showed complete marrow remission after antibody treatment as described. Patient no. 8 had foci of immature ganglioneuroma cells in the skin tumor nodules at the time of entry into the

study. Although his skin nodules did not change in number or size after antibody treatment, nodules removed showed fully mature ganglioneuroma cells. Patient no. 9 had more than 90% of his marrow replaced by neuroblastoma. Ten days after treatment, there was > 80% reduction in the tumor count both by Wright staining, as well as by 3F8 immunofluorescence. He also showed improvements in his peripheral neutrophil count. Patient no. 15 showed tumor necrosis in his bone marrow biopsies 2 weeks after treatment, which persisted for 6 weeks. Patient no. 16 showed continual decrease in the percent of G_{D2} positive tumor cells in her marrow from 5% before treatment to 1.5% at 1 week and 0.03% at 4 weeks after treatment. Patient no. 17 also showed tumor necrosis in his bone marrow biopsy 4 weeks after antibody therapy. However, patients no. 15, 16, and 17 showed no change in the degree of tumor infiltration of their bone marrows by routine histology.

Relationship Between Tumor Response and Prior Therapy

Among the four melanoma patients who responded to 3F8 (PR or MR), three had no prior systemic therapy and one had high-dose therapy followed by autologous bone marrow reinfusion. Among the five nonresponders, two had prior chemotherapy and one (no. 7) had circulating anti-mouse antibody at the time of 20 mg/m^2 3F8 infusion. This patient was sensitized to mouse IgG after the imaging dose injected 5 weeks before treatment. Although the remaining two patients had no prior systemic therapy, they both had rapidly progressive metastases in bone (both), bone marrow (both), and brain (one) that necessitated palliative radiation at the time of treatment. The two neuroblastoma patients who had major responses, (no. 1 and no. 3), had undergone allogeneic BMT before antibody therapy. Patient no. 13 also had allogeneic transplantation. However, she had rapidly progressive and widespread disease at the time of antibody treatment.

Relationship Between Tumor Response and the Presence of G_{D2} in Tumor Assayed by 3F8 Binding Before Antibody Therapy

Among the melanoma responders (no. 5, 6, 10, and 15), two patients (no. 5 and 6) did not show uptake of radiolabeled 3F8. However, in vitro they showed 100% 2+ and 20% 2+ staining with 3F8, respectively. Patient no. 10 had 30% 3+ and patient no. 15 had 20% 3+ staining. Among the nonresponders, in vitro staining ranged from 10% 3+ to 50% 3+. Four of the five patients showed tumor uptake by 3F8 radioimaging.

Among the neuroblastoma patients, all had strong (80% to 100% 3 to 4+) in vitro binding to their tumor and consistent uptake of 3F8 in vivo.

DISCUSSION

3F8, a murine IgG_3 MoAb, was administered IV to 17 patients with metastatic neuroblastoma or melanoma in a phase I clinical trial. Various side effects occured, but were generally tolerable and reversible. Pain, hypertension, urticaria, and decreases in serum complement levels occurred at all dosages of 3F8, and temporally associated with antibody infusion. Febrile reactions, nausea, mild diarrhea were seen, more frequently at higher doses. All side effects were controlled with symptomatic therapy such as analgesics, antipyretics, and slowing of the infusion rate. Patients observed past 6 months have not shown any long-term side effects, including changes in skin pigmentation, and peripheral or central nervous system.

Peak serum 3F8 levels were related to the amount of antibody infused. However, the human anti-mouse response did not increase with the 3F8 dose received. Patients with significant circulating human anti-mouse antibodies had minimal side effects and also no therapeutic benefits from 3F8 injections. Those patients with < 1,000 U/mL of anti-mouse antibody experienced many of the observed side effects as well as therapeutic responses from 3F8 injections. It is possible that a critical component of the anti-mouse response may have been anti-idiotypic and that its intensity may vary among patients. Nevertheless, ten of 17 patients in this study had peak anti-mouse antibody levels of < 1,000, and might benefit from repeated 3F8 injections.

Major antitumor responses were noted in four of 17 patients. These included metastatic sites in bone marrow, bone, lymph nodes, and liver. Two of these patients had neuroblastoma and two had melanoma. Other patients with minor or mixed responses included subcutaneous, cutane-

ous, and muscular metastases. The duration of response was variable (4 weeks to 63 weeks). Neuroblastoma tissue stained homogeneously with the antibody 3F8, probably because of the uniform possession of the antigen G_{D2}. However, malignant melanomas demonstrate variable expression of G_{D2} among patients and between tumor sites in the same patients as previously reported.[13] This finding might explain why CRs were observed in neuroblastoma and not in melanoma patients.

Major antitumor responses were seen in patients who had no prior systemic chemotherapy, or who had undergone allogeneic BMT. It is possible that the graft-*v*-host reaction (GVH) in patients no. 1 and 3 may have contributed to the antitumor response, even though by itself GVH was insufficient. There were two melanoma patients with advanced bone and marrow metastases who had no prior chemotherapy and who did not show objective antitumor response to 100 mg/m^2 of 3F8. Since this series of patients is small and heterogeneous, the impact of the extent of disease, antibody dose, and prior therapy on the magnitude of tumor response remains to be determined.

A number of mechanisms may be important for the biological effects of the antibody 3F8 in patients. 3F8 activates human complement[8] and mediates ADCC with human WBCs.[15] Using immunohistological techniques, it has been shown that in normal human tissues, the antigen G_{D2} is highly restricted to neurons and peripheral pain fibers.[16] Previous studies using radiolabeled 3F8 have shown that it does not cross the intact blood brain barrier in mice and humans.[11,13] In the imaging studies of 14 patients, no uptake was seen in brain or spinal cord tissues, and none of the patients demonstrated long-term neurological deficits. The reactivity of pain fibers with 3F8 may explain the pain reactions that accompany 3F8 infusions. Whether this pain reaction is mediated through complement activation or other cytotoxicity mechanisms remains to be elucidated. Tumor samples obtained after 3F8 infusion are currently being examined for infiltration by immune cells and complement components. Understanding the effector mechanism operative in these patients may facilitate therapeutic modulation by other biological response modifiers in future studies.

Most MoAbs reported to date have been primarily useful for in vitro diagnosis. When administered to patients, the majority did not demonstrate antitumor effects.[17] The ability to effect major tumor responses with a small dose of monoclonal antibody is uncommon.[2-5,18,19] Some have suggested that the usefulness of MoAbs may be best realized in patients with minimal disease. All the patients in our phase I study had gross metastatic disease, many with disease in bone and bone marrow. However, more than a third of these patients experienced antitumor responses. Given the reversibility of all the side effects during treatment, the antibody 3F8 is potentially useful in the selective eradication of microscopic metastatic disease in the hope of achieving longer survival.

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