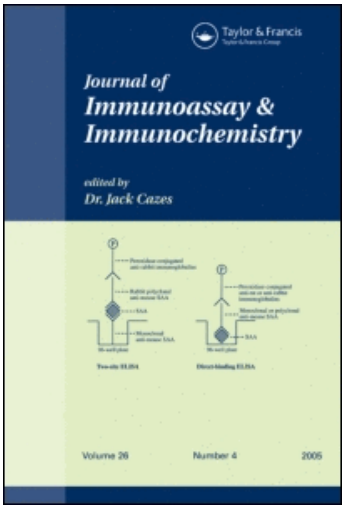

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METASTATIC UVEAL MELANOMA: AN OCULAR MELANOMA ASSOCIATED ANTIGEN IN THE SERUM OF PATIENTS WITH METASTATIC DISEASE

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(KEY WORDS: double-determinant immunoassay, metastatic melanoma, monoclonal antibody, ocular melanoma-associated antigen, serum)

ABSTRACT

Two monoclonal antibodies, MAb8-1H and ME491, which bind to different determinants of the same highly glycosylated melanoma-associated antigen, were used to determine melanoma-associated antigen levels in serum samples from patients treated for primary choroidal or ciliary body melanoma and who subsequently developed systemic metastasis. An immunoassay was developed in which ME491 was absorbed to polystyrene beads in order to bind the melanoma-associated antigen present in serum. ^{125}I-MAb8-1H was used to detect the bound antigen. This double-determinant immunoassay is both sensitive and reproducible. Supernatant fluids of tissue cultured melanoma cell lines served as positive standards for the calculation of melanoma-associated antigen units. The mean serum levels of melanoma-associated antigen were 7.7 units for patients with benign ocular conditions, 9.51 units for patients with choroidal melanoma without documented metastatic disease, and 48.3 units for patients with choroidal melanoma and documented systemic metastasis. The clinical implications of using this test as a screening method for the detection of metastatic disease is discussed.

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INTRODUCTION

Malignant melanoma cells, both in vivo and in vitro, characteristically release, or shed, melanoma-associated antigens (MAAs) into the environment. Several MAAs, such as the proteoglycan antigen, have been identified and measured in the serum of patients with cutaneous melanoma and have been found to correlate with the tumor burden of the patient during the clinical course of the disease (1-3).

Monoclonal antibodies (MAbs) MAb8-1H and ME491 define different epitopes of a highly glycosylated melanoma-associated antigen (MAA) present in both ocular (4,5) and cutaneous (6) melanomas. The MAA, characterized as a mucin-type glycoprotein, is shed by tissue culture melanoma cells into the surrounding media. On the basis of this finding, we evaluated the sera from patients with ocular melanoma with and without documented metastatic disease for the presence of the MAA identified by MAbs MAb8-1H and ME491 in a sensitive double-determinant immunoassay (DDIA) and compared the results with the clinical course of the patient.

MATERIALS AND METHODS

Patients' Sera

The 167 serum samples used in this study were stored frozen at -70°C prior to use. Sixty-five serum samples were obtained from patients without choroidal melanoma prior to cataract extraction (control group); 86 serum samples were from

patients treated for chorcidal melanoma by enucleation or cobalt plaque radiotherapy without documented metastatic disease; and 17 serum samples were from patients with documented metastatic disease following treatment of the primary melanoma. Serum gamma glutamyl transpeptidase (GTP) values were obtained from the patients' records.

MAbs

The production and characterization of MAb8-1H and ME491 have been described previously (4,6). MAbs were purified from ascites fluid by 50% ammonium sulfate precipitation and by gel permeation chromatography (4,6).

Cell Lines

The SK-MEL-23 melanoma cell line, maintained in supplemented serum-free medium (SSFM), was used as a source of control MAA in the binding studies and has been described previously (6).

Radioimmunoassay

MAb8-1H was radioiodinated by the iodogen method to a specific activity of 2-4 uCi/ug (4). The DDIA utilized to measure MAA in the patients' sera has been described in detail previously (7). Briefly, polystyrene beads (6.35 mm; Precision Plastic Ball Co., Chicago, IL) were incubated with ME491 (1:1000 dilution of ascites fluid), referred to as the catcher MAb, overnight at 4°C. The beads were washed three times with phosphate-buffered saline (PBS), incubated with PBS containing 2% bovine serum albumin

PBS/BSA) for one hour, and then transferred to reaction trays (Centocor, Malvern, PA) containing 200 ul serum diluted in calcium- and magnesium-free PBS and supplemented with PBS/BSA. After overnight incubation at 4° C, the beads were washed with PBS using Pentawash II (Abbott Laboratories, Chicago, IL), and 200 ul of ^{125}I-MAb8-1H (100,000 cpm), referred to as the detector MAb, was added. After overnight incubation, the beads were washed and the bound radioactivity was measured in a gamma spectrometer.

All assays were performed in duplicate or triplicate, depending on the amount of the patient's serum available. Cpm of beads incubated with PBS only were subtracted from test sample cpm. Units of MAA were calculated from a standard curve derived by using SK-Mel-23 SSFM and diluted 1:1 to 1:16 in normal human serum. The cpm of beads corresponding to a 1:4 dilution of SSFM was defined as 100 units. A standard curve was included in all assays. Groups were compared using the Student's t-test. Differences were considered statistically significant for $p < 0.05$.

RESULTS

The mean serum concentrations of MAA detected by the DDIA for the control group and for patients treated for primary uveal melanoma without documented metastatic disease was 7.7 and 9.5 DDIA units respectively. Each differed significantly ($p < 0.001$) from values obtained from patients treated for primary uveal melanoma with documented metastatic disease (48.3 DDIA units), but did not differ significantly from each other. Table 1 gives the distribution of DDIA units among the patient groups.

TABLE 1

Detection of MAA in Sera of Patients as Determined in DDIA Using ME491 and ^{125}I-MAb8-1H

	Number of Sera at the Following DDIA Units								
GROUPS:	0-10	10-20	20-30	30-40	40-50	50-100	>100	Total# of Sera	% sera > 20 DDIA units**
Control	51*	9	1	1	0	3	0	65	8%
Choroidal melanoma without documented metastasis	62	13	4	3	2	0	1	85	12%
Choroidal melanoma with documented metastasis	4	1	0	1	2	9	0	17	71%

* The number of sera giving a response of 0-10 DDIA units.
** Rounded to nearest percent.

Patients treated for choroidal melanoma with metastasis were more likely to have elevated levels (> 20 DDIA units) of circulating MAA ($p < 0.001$). Eight percent of control patients, 12% of choroidal melanoma patients without documented metastasis, and 71% of choroidal melanoma patients with documented metastatic disease have serum MAA levels above 20 DDIA units.

Serial blood samples, drawn at approximately every three to six months, were available for 13 patients treated for a choroidal melanoma who subsequently developed systemic metastases. In four of these patients sera was obtained before

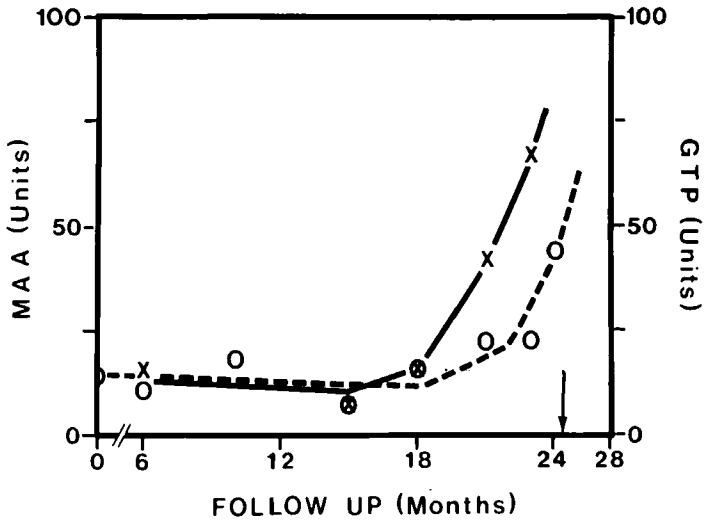

Figure 1. Time course showing changes in serum MAA (-x-x-x-) and GTP (-0---0---0-) in a patient in whom liver metastasis developed. Arrow indicates time following enucleation at which liver scan became abnormal, followed by positive liver biopsy.

treatment and at regular intervals, therafter, until the documentation of metastatic disease. The serum concentration of MAA was compared to serum liver enzymes and the clinical course of these four patients. In one of these four patients, elevated MAA levels (41.5 units) were recorded prior to elevated serum liver enzyme (GTP) or the finding of an abnormal liver scan. This patient was a 60-year-old man evaluated in February 1981 for a pigmented lesion of the right eye. The clinical diagnosis was choroidal melanoma and the eye was treated by cobalt plaque radiotherapy. The patient was seen regularly in follow-up visits, and in December 1982

clinical evaluation and serum liver enzymes indicated no metastatic disease, despite elevated serum MAA levels (Figure 1). Because ophthalmic evaluation at this time indicated new tumor growth, an iridium plaque was applied to the right eye. Approximately three months later, metastatic disease was documented by liver biopsy following an anormal liver scan coincident with elevations of serum liver enzymes. The patient expired shortly thereafter. In the remaining three patients serum MAA became elevated at approximately the same time as other liver function tests became abnormal.

DISCUSSION

Most patients who die of metastatic uveal melanoma die within two years following ocular surgery (8). The majority of these patients have liver metastasis, which is often first indicated by abnormal liver function tests (8,9). In this study we have used a sensitive DDIA to demonstrate significant elevations of serum MAA levels in patients with choroidal melanoma and documented metastatic disease as compared to those levels in choroidal melanoma patients without metastatic disease or patients with benign ocular disorders. Using a DDIA similar to that used in the present study, Ross and associates (1) found elevated levels of circulating proteoglycan antigen, another MAA, in 76% of cutaneous melanoma patients with a high metastatic tumor burden, as compared to 22% of patients with light tumor burden and 2% of healthy donors. In our study, we found statistically significant levels of elevation of MAA in

the sera of patients with documented metastatic disease ($p < 0.001$). With regard to patients with ocular melanoma, the predictive value of the test was 55% with false positive rate of 9%. The false negative rate for this test for patients with documented metastatic disease was 29% with a sensitivity of 71%. These values suggest that this test may have some, but limited value as a screening test for the detection of metastatic disease.

It would be useful to detect subclinical metastasis in patients with ocular melanoma, since once the diagnosis of metastasis has been established, the duration of survival is usually less than on year (9, 10). Serial blood samples were obtained before treatment and serially, thereafter, until the documentation of liver metastasis in four patients. Serum MAA levels of three of these patients became elevated coincident with other indicators of hepatic dysfunction. In one patient, however, the serum MAA level was elevated (41.5 units) prior to abnormal serum liver enzymes or liver scan (Figure 1). These results suggest that this test may not offer significant advantages over conventional testing procedures in detecting early metastatic disease; however, additional serial blood studies are needed in order to more fully assess the feasibility of utilizing this test in the detection of early subclinical metastatic disease.

The MAA detected by ME491 and MAb8-1H, a glycoprotein found in some normal tissues, may be increased in various diseases other than cancer, so that detection of elevated serum MAA levels of

some control patients or patients with choroidal melanoma without other clinical evidence of metastasis (Table 1) is not altogether unexpected. Hatae and associates (11) measured circulating levels of a cancer-related glycoprotein (CRG) and found that patients with infectious disease processes had mean CRG levels twice that of normal controls, but still less than patients with metastatic disease ($p < 0.02$). Future investigators measuring MAAs by the technique described in this report should also be aware of these potential sources of error.

Bolmer and Davidson (12) have reported in patients with various malignancies high serum levels of a cancer-associated glycoprotein which correlated with the stage of disease and the tumor burden of the patient. Furthermore, they isolated, purified, and characterized a 50,000 molecular weight glycoprotein from the serum of patients with malignancy. Interestingly, the glycoprotein contained unusually high levels of the amino acids serine, glutamic acid and glycine, and other amino acids in similar proportion to the MAA detected by MAb8-1H and ME491 in this study. Whether the cancer-associated antigen described by Bolmer and Davidson (10) is identical with the MAA described here warrants further investigation.

The identification of other MAAs shed into tissue culture media, such as the MAA detected by MAb8-1H and ME491, may provide additional MAAs useful in the detection of early subclinical metastasis in patients with primary uveal melanoma. Such studies are currently under consideration in our laboratory.

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