SKIN TESTS WITH SOLUBLE MELANOMA ANTIGENS IN PATIENTS WITH CHOROIDAL TUMORS

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The cutaneous delayed hypersensitivity reactions with a soluble melanoma antigen in patients with choroidal mass lesions were studied. Ninety percent of patients with pathologically documented choroidal melanomas had positive melanoma antigen skin test responses. There did not appear to be any difference in the histologic appearance of the tumor nor in the disease status of those patients with positive versus those patients with negative skin test reactions. A 21% instance of false-positive responses with this partially purified soluble melanoma antigen in patients with simulating lesions was observed. The cause for this reactivity is unclear; however, from previous work tissue-associated antigens or fetal antigens are the most probable etiologies for the false-positive melanoma antigen skin tests observed. Further purification of the melanoma-associated antigen preparation may increase the specificity. The results of this study would mitigate against the use of this soluble melanoma antigen skin test in the primary evaluation of patients with pigmented choroidal mass lesions. Currently, the assay is being tested to ascertain its correlation with prognosis and as a means of monitoring immunotherapy.

Cancer **40:1650-1654, 1977.**

THE CLINICAL DIFFERENTIATION OF CHOROIDAL TUMORS may be difficult.[10,16,17] We and others have previously described the existence of tumor-associated antigens in choroidal melanoma.[5,6,9,22] In a preliminary study we reported that the cutaneous delayed hypersensitivity reaction with a soluble melanoma antigen could be used to discriminate patients with primary ocular malignant melanomas from patients having benign and metastatic simulated lesions.[5] Since our initial report, we have tested a larger number of patients to determine the specificity and sensitivity of this assay, and its correlation with prognostically useful histopathologic parameters.

* Department of Ophthalmology, University of California, San Francisco, California. Supported in part by the Cancer Research Coordinating Committee, University of California, Berkeley, California, Grant #75 SF11; and Fight for Sight, Inc. G-574, New York, New York.

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The authors thank the Members of the Ocular Oncology Group and physicians who have kindly allowed them to skin test their patients on referral, and the following people for their technical assistance: O. B. Lee, K. Tanner, B. Queen, P. Jones, S. Trauner, and J. Brunn.

Accepted for publication February 15, 1977.

MATERIALS AND METHODS

Subjects

All subjects were evaluated at the Ocular Oncology Clinic of the University of California, San Francisco, after referral because of suspected ocular melanoma; they had a routine opthalmological workup for this disease, which included: fundus photographs or drawing; transillumination; Goldmann perimetry; fluorescein angiography; and when clinically indicated, ultrasonography and the radioactive phosphorous uptake test.

At the time of this report subjects who were skin tested can be categorized into three groups: 71 patients have had histopathologic confirmation of their ocular malignant melanoma; 28 patients are believed, after extensive clinical examination, to have simulating choroidal lesions either of benign nature or due to metastatic tumor deposits; and 23 patients have the clinical diagnosis of a choroidal melanoma, but have not had histopathologic comfirmation of this diagnosis.

All skin testing was performed by one of us (DHC). All were given oral and written explanation of our experimental protocol and informed consent was obtained.

Skin Test Materials

The details of the methodology used in antigen preparation have previously been described.[5,12,13] All of the tests were performed from one extract prepared from metastatic mela-

noma tissue, obtained in a sterile manner from a patient's liver at autopsy. A crude membrane extract was prepared, sonicated, and partially purified by column chromotography using a Sephadex G-200 column. The second of three protein peaks eluted off the column was used as the soluble melanoma skin test antigen. This material had a molecular weight of approximately 38,000. Its sterility was ensured by Millipore filtration and culture in blood agar and thioglycolate broth. For skin tests this material was adjusted to a protein concentration of 39 μg/0.1 ml, dispersed into syringes, and stored at −70°C until it was used. Standard recall antigens used in this study were mumps (Eli Lilly & Co., Indianapolis, Indiana), SKSD (40 units streptokinase, 10 units streptodornase, Lederle Laboratories, Pearl River, New York) and dermatophytin "O" (Hollister-Stier Laboratories, Spokane, Washington).

Skin Test Procedure

Patients received intradermal inoculations of standard recall antigens and the soluble melanoma antigen in the interscapular region. The diameter of induration of all skin tests was measured at 48 hours using a Vernier caliper. A positive soluble melanoma antigen skin test was defined as greater than 6.5 mm of induration, and a positive standard recall antigen skin test as greater than 5 mm of induration at 48 hours. In previous tests done with tumor-derived antigens and the standard recall antigens we have biopsied positive reactions. Histologically, these consisted of perivascular infiltration of mononuclear cells in the upper dermis. Positive reactions to tumor-derived and standard recall antigens were indistinguishable on pathologic exam.[7]

RESULTS

One hundred and twenty-two patients were skin tested with standard recall antigens and the soluble melanoma antigen. All patients with choroidal melanomas had positive cutaneous delayed hypersensitivity reactions with at least one standard recall antigen, and there was no difference in reactivity between patients with ocular melanoma and normal subjects with the standard recall antigens. Seventy-one patients with histologically documented melanomas were skin tested either preoperatively (57), within 6 months of enucleation (9) or greater than 6 months after enucleation (5). Sixty-two of these patients had positive skin test reactions with the soluble melanoma antigen (Table 1), and there was no difference in the incidence of positive reactions between groups of patients tested at different times. Nine patients with histologically confirmed melanomas had negative skin test reactions (seven preoperative, two postoperative). None of these patients have developed metastatic disease on follow-up examination. Two patients in our series with choroidal melanomas have developed metastatic disease. One had a positive melanoma antigen skin test at the time metastatic disease was documented and the other had a positive test 2 months prior to the development of metastises. All 23 patients who have a clinical diagnosis of ocular melanoma gave positive skin tests. Most of these patients have been treated with alternate modes of therapy other than enucleation (xenon photocoagulation, cobalt plaques, or iridocyclectomy;) some are being clinically observed until growth of their tumor is documented.[4] Four patients are being followed in whom the diagnosis of a choroidal melanoma is uncertain, however, it is considered most likely (data not in Table 1).

TABLE 1. Soluble Melanoma Antigen Skin Tests

Disease category	Positive/Total (%+)	Mean induration
Pathologically confirmed melanomas	62/71 (87)	12.2 ± 4.3*
Simulating lesions	6/28 (21)	5.9 ± 3.9
Benign	(4/20)	
Metastatic	(3/8)	
Clinically diagnosed melanomas	(23/23) (100)	12.9 ± 2.3

* Statistically significant difference: pathologically confirmed melanomas and simulating lesions ($p < 0.001$).

In 28 patients originally referred to our Oncology Unit with the diagnosis of a choroidal melanoma, subsequent extensive clinical examination and follow-up have demonstrated that these patients have simulating lesions (Table 2). Three of six patients with choroidal hemangiomas have had positive skin tests with the soluble melanoma antigen, as have two out of six patients tested who have metastatic simulating lesions with primary malignancies elsewhere in the body. One additional patient with a histologically confirmed benign retinal lesion has also had a positive soluble melanoma antigen skin test.

Patients with ocular melanomas had a statistically significant increased incidence of positive reactions and area of induration with the soluble

TABLE 2. Skin Tests in Patients with Simulating Lesions

Disease category	Number of positive/number tested
Benign lesions	
Choroidal Nevi	0/6
Choroidal hemangioma	3/6
Miscellaneous lesions (choroidal detachment, diffuse uveitis, iris cyst, melanacytoma, retinal pigment epithelial hyperplasia, Jensen's peripapillary choroiditis, melanosis and fibrocytoma of the retina).	1/8
Metastatic lesions	0/4
Breast carcinoma	0/4
Thyroid carcinoma	0/1
Lung carcinoma	1/2
Metastatic adenocarcinoma	1/1

melanoma antigen versus patients with simulating lesions ($p < 0.001$.) In four patients with positive skin tests and histologically confirmed melanomas repeat testing was done. In three patients the skin test reactivity did not change, and in one the skin test became negative. In the latter patient there was no change in clinical status, however, he was retested approximately 2 years after therapy. In previous studies, repeat skin testing did not produce sensitization to these tumor antigens.[7]

In a coded manner microscopic sections of 23 patients choroidal melanomas were ranked on the basis of prognostically useful histologic pathologic parameters.[2,3,23] The rank order based on histologic parameters was compared with the rank order based on the diameter of induration with the soluble melanoma antigen. Using Spearman analysis there did not appear to be any correlation between histopathologic correlates of malignancy and the size of the skin test reaction with the soluble melanoma antigen in the 23 patients studied in this manner (Rs 0.07). The mean skin test result with spindle B melanomas was 11.5 ± 4.5 SD with mixed cell melanomas it was 12.6 ± 4.9 SD.

DISCUSSION

We have studied the specificity, sensitivity, and correlation with histopathology of a soluble melanoma antigen skin test in a group of patients with suspected choroidal melanomas.

Ninety percent of patients with pathologically documented choroidal melanomas had positive melanoma antigen skin tests reactions. There did not appear to be any difference in the histologic appearance of the tumor nor in the disease status of those patients with positive skin test reactions versus patients with negative skin test reactions.

We observed a 21% incidence of false-positive responses with this partially purified soluble melanoma antigen tested in an identical manner as previously reported.[5] The cause for this reactivity is unclear. Because of the need for a large amount of melanoma antigen to test this large group of patients, all work was done using crude Sephadex fractions of an allogeneic tumor metastatic to the liver. Unfortunately, the amount of tumor available for processing from primary choroidal melanomas precludes autologous testing. In previous studies using Sephadex fractions some cross-reactivity has been observed in patients with cancers other than melanoma (manuscript in preparation.) From previous work it appears necessary to separate these Sephadex fractions further by gradient polyacrylamide gel electrophoresis to obtain purified, tumor-associated antigens that are not contaminated by fetal or tissue associated components.[11,12] On the basis of previous work we think it is unlikely that a bacterial or HLA antigen was responsible for the false-positive reactions observed.[5,7] Because of the question of safety, we have processed mouse leukemia cells induced by Rauscher leukemia virus in an identical manner as a soluble melanoma antigen. After such processing, we were unable to detect by *in vivo* or *in vitro* techniques the presence of infectious virus (unpublished observation).

There are two probable etiologies for the false-positive melanoma antigens skin tests observed: one, the quantitative nature of a positive versus a negative result; and two, cross-reacting tissue-associated antigens. We have previously defined a positive soluble melanoma skin test reaction as greater than 6 mm of induration at 48 hours based on the desired clinical discrimination. In biopsy of clinically negative standard recall antigen skin tests (2–3 mm of induration) we have also observed histopathologic parameters of a delayed hypersensitivity reaction. Since the difference between a positive and negative skin test result is only quantitative in nature, it is conceivable that some false-positive reactions are observed. Malignant melanoma is made up of both neoplastic cells and normal supporting stroma. It is likely that this supporting stroma and also melanoma cells themselves may have

tissue antigens, and that these normally occuring antigens may produce sensitization and thereby be responsible for the false positive responses observed in patients with simulating lesions. In previous studies with different types of tumor-associated antigen preparations, a marked difference in the sensitivity and the incidence of false positive reactivity has been noted. While there appears to be a trend towards greater sensitivity and specificity with the more purified antigens, this has not been universal.[1,5,7,8,12–15,18–21] Currently, we are studying more highly purified soluble melanoma antigen preparations made from a variety of primary metastatic tumors to investigate this issue further.

Historically, 20% of eyes enucleated with the diagnosis of a choroidal melanoma were found on pathological examination to have other simulating lesions;[10,17] however, in centers specializing in ocular oncology, the incidence of incorrect diagnoses is now less than 2%.[4] The results of this study would mitigate against the use of this test in the routine evaluation of patients presenting to their opthalmologists with choroidal mass lesions. While the incidence of the false-positive reactions (21%) can probably be lowered using more purified melanoma-associated antigen preparations, at least two other factors make it unlikely that this cutaneous delayed hypersensitivity assay can be used on a large scale. To prepare polyacrylamide gel electrophoresis fractions for skin testing a great volume of melanoma material must be obtained, and the logistics of antigen preparation on a large scale are quite difficult. Safety is a second major issue. The molecular weight of these soluble melanoma antigens (80,000) makes it highly unlikely that any intact viral particles or nuclear information are present. As mentioned previously, we have processed virally induced animal tumors in a similar manner as the soluble melanoma antigen and have been unable to identify an infective virus. To date we have not seen any untoward effects in approximately 1500 skin tests of patients with crude membrane or soluble tumor extracts. However, since we have avoided testing these materials in normal subjects we cannot say with certainty that there is no transmission of low molecular weight oncogenic information. While there is no indication of horizontal transmission in man with these skin test materials, we do not think it is justified to use this material on any subjects who are unlikely to have a malignant process. Probably with the improved clinical diagnostic techniques in choroidal melanoma detection, the use of these skin tests would have little diagnostic value. We are currently using this type of assay to study patients with choroidal melanomas serially to determine whether the results of the skin test is of prognostic importance and is useful in monitoring choroidal melanoma patients receiving immunotherapy.

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