Analysis of the Antigenic Profile of Uveal Melanoma Lesions with Anti-Cutaneous Melanoma-associated Antigen and Anti-HLA Monoclonal Antibodies

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Abstract

The reactivity of 12 surgically removed uveal melanoma lesions with monoclonal antibodies (MoAb) to -membrane-bound and 2 cytoplasmic cutaneous melanoma-associated antigens (MAA), to the 2 subunits of HLA Class I antigens and to the gene products of the HLA-D region was compared with that of cutaneous melanoma lesions and correlated with their histotype. The membrane-bound determinants defined by the anti-M, 92,000 and 45,000 MAA MoAb TP39.1, anti-M, 110,000 MAA MoAb M111, anti-M, 118,000 MAA MoAb TP26.1, anti-M, 115,000 MAA MoAb 345.134, anti-ICAM-1 MoAb CL203.4 and anti-M, 31,000 MAA MoAb M2590, and the cytoplasmic determinants defined by the anti-MAA MoAb 456.12 and 2G-10 display a distribution in uveal melanoma lesions similar to that in cutaneous melanoma lesions. On the other hand, the membrane-bound determinants defined by the -M, 100,000 MAA MoAb 376.96, anti-9-O-acetylganglioside MoAb MES11 and anti-GD3 ganglioside MoAb ME361 were not detected in the uveal melanoma lesions tested. Furthermore, the membrane-bound determinants defined by the anti-GD3 MoAb R24, anti-serve growth factor receptor MoAb ME20.4, anti-M, 97,000 MAA MoAb 140.240, anti-carci-noembryonic antigen MoAb B1.1 and anti-HMW-MAA 149.53 and 225.28, and 763.74 have a markedly lower expression in uveal than in cutaneous melanoma lesions. Incubation of uveal melanoma lesions with the pool of the MoAb 149.53, 225.28, and 763.74 recognizing distinct and spatially distant determinants of the HMW-MAA increased the intensity of staining of six lesions and stained four lesions which were not stained by the individual monoclonal antibodies. The distribution of HLA Class I antigens in uveal melanoma lesions resembles that in cutaneous melanoma lesions, since they are expressed in all the lesions of the mixed and epithelioid type but were not detected in those of the spindle type, i.e., the counterparts of neovascular nevi. HLA Class II antigens are expressed with a lower frequency in uveal than in cutaneous melanoma lesions, since they were detected only in 2 of the 12 lesions. One of them is of the mixed type and the other one of the epithelioid type. Besides HLA antigens the determinants defined by the anti-carci-noembryonic MoAb B1.1, anti-ICAM-1 MoAb CL203.4, and anti-GD3 MoAb R24 displayed a differential distribution in the different histotypes of uveal melanoma, since they are preferentially expressed in lesions of the mixed and epithelioid type.

Introduction

A large number of monoclonal antibodies to MAA1 have been developed utilizing mice immunized mainly with cell lines originated from metastases spread from cutaneous melanoma lesions (for review, see Refs. 1 and 2). These reagents in conjunction with anti-HLA monoclonal antibodies have been extensively utilized to characterize the antigenic profile of primary and metastatic cutaneous melanoma lesions (for review, see Refs. 1–4). On the other hand, a limited number of monoclonal antibodies have been elicited with melanoma cells isolated from surgically removed ocular melanoma lesions. Donoso et al. (5) described a murine monoclonal antibody which identifies a M, 40,000–50,000 glycoprotein expressed both by cutaneous and uveal melanoma cells. Damato et al. (6) raised 5 rat monoclonal antibodies to uveal melanoma cells. Testing with a limited panel of targets identified one monoclonal antibody which reacted with 12 uveal melanoma lesions but with none of the controls. This antibody identified a protein doublet in the molecular weight range of 55,000. Staining of surgically removed lesions with the murine (7) and the rat (6) monoclonal antibodies demonstrated significant variations in the antigenic profile of uveal melanoma cells.

A number of uveal melanoma lesions have been tested with a small panel of anti-cutaneous MAA monoclonal antibodies (8–10). There is general agreement that, like cutaneous melanoma lesions (11, 12), uveal melanoma lesions are heterogeneous in the expression of determinants recognized by monoclonal antibodies. On the other hand, conflicting data have been reported about the distribution of cutaneous MAA in uveal melanoma lesions. van der Pol et al. (9) have stained 25 uveal melanoma lesions with monoclonal antibodies to 7 cutaneous MAA and have found that the anti-HMW-MAA MoAb 225.28 and the anti-GD3 MAA MoAb R24 stained a markedly lower percentage of uveal than of cutaneous melanoma lesions. In contrast, Bomanji et al. (10) have reported that the MoAb 225.28 and 763.74 to distinct determinants of the HMW-MAA and the anti-M, 100,000 MAA MoAb 376.96 stained the large majority of the uveal melanoma lesions tested. The expression of HLA antigens in uveal melanoma lesions has been investigated only by Jager et al. (13). They stained 56 paraffin-embedded uveal melanoma lesions with anti-HLA monoclonal antibodies and detected HLA Class I antigens in 47 lesions and HLA Class II antigens in a markedly lower number of lesions. No relationship was found between expression of HLA antigens and cell type of melanoma, tumor size, and patients’ survival.

No information is available about the expression of β2-microglobulin, the light chain of HLA Class I antigens, and of HLA-DR, -DQ, and -DP antigens in uveal melanoma lesions.

Materials and Methods

Monoclonal Antibodies and Conventional Antisera. The isotype and the specificity of the monoclonal antibodies used are listed in Table 1.
The MoAb TP36.1 immunoprecipitates a membrane-bound glycoprotein with apparent molecular weights of 92,000 and 45,000. The reactivity of the MoAb TP36.1 and TP39.1 was described elsewhere are secreted by hybridomas containing the corresponding references. The MoAb TP36.1 and TP39.1 showed that MoAb M2590 reacts with pigmented choroidal melanoma with the panel of anti-cutaneous MAA monoclonal antibodies showed that MoAb M2590 reacts with pigmented choroidal cells, MoAb TP39.1 stains the retinal pigmented epithelium, with the corresponding references. The MoAb TP36.1 and TP39.1 which will be described elsewhere are secreted by hybridomas constructed with splenocytes from a BALB/c mouse immunized with immune interferon-treated cultured human melanoma cells (Colo 38). The MoAb TP36.1 immunoprecipitates a membrane-bound glycoprotein with the apparent molecular weight of 118,000; the MoAb TP39.1 immunoprecipitates a dimer which consists of two components with apparent molecular weights of 92,000 and 45,000. The reactivity of the monoclonal antibody preparations used in the present investigation was monitored by testing with known positive tissue substrates. Fluorescein isothiocyanate-conjugated rabbit anti-mouse immunoglobulin antibodies were purchased from Cappel Laboratories (Cochrane, PA). The reagent had a fluorescein:protein molar ratio of 3 and was used at a protein concentration of 0.5–1 mg/ml. Reagents for indirect immunoperoxidase (ABC Kit) were purchased from Vector Laboratories (Burlingame, CA).

### RESULTS

Reactivity of Uveal Melanoma Lesions with Anti-Cutaneous MAA Monoclonal Antibodies. Staining of normal ocular tissues with the panel of anti-cutaneous MAA monoclonal antibodies showed that MoAb M2590 reacts with pigmented choroidal cells, MoAb TP39.1 stains the retinal pigmented epithelium,
MoAb TP36.1 stains the pigmented epithelium of the ciliary body, MoAb 345.134 and 376.96 recognize the pigmented epithelium of the ciliary body and the corneal epithelium, and MoAb CL203.4 decorates the endothelium of capillaries and small vessels. No staining of normal ocular tissue was detected with the remaining anti-MAA monoclonal antibodies included in the panel.

The results of immunohistochemical staining with anti-MAA monoclonal antibodies of uveal melanoma lesions of different histiotypes are summarized in Tables 2 and 3. Representative staining patterns are shown in Fig. 1. The following points are noteworthy: (a) no convincing staining was obtained with the MoAb 376.96; (b) the MoAb 345.134 stained all the lesions homogeneously; (c) the MoAb CL203.4 displayed a preferential reactivity with uveal melanoma lesions of the mixed and epithelioid type; (d) the MoAb 149.53, 225.28, and 763.74 stained only five lesions without any relationship to their histiotype. The pattern was homogeneous in two lesions of the epithelioid type with a dull intensity in one. Furthermore, the staining was restricted to isolated areas in one epithelioid and in one mixed type lesion and was heterogeneous in another mixed type lesion. Testing of the lesions with a pool of the three anti-HMW-MAA monoclonal antibodies increased the intensity of staining and changed the pattern from heterogeneous to homogeneous in three lesions and from isolated areas to heterogeneous staining in a fourth lesion. Furthermore, staining with the pool of the three monoclonal antibodies detected the HMW-MAA in four lesions which had not been stained by the individual anti-HMW-MAA monoclonal antibodies. The intensity of the staining of one lesion which was stained with an homogeneous pattern by individual anti-HMW-MAA monoclonal antibodies was increased when the lesion was tested with the pool of anti-HMW-MAA monoclonal antibodies. Two lesions which were not stained by individual anti-HMW-MAA monoclonal antibodies were also not stained by the pool of the three monoclonal antibodies (Table 4); (e) among the antiganglioside monoclonal antibodies only the MoAb R24 reacted with uveal melanoma lesions, the staining was restricted to isolated areas of three lesions of the mixed type and of one of the epithelioid type; (f) the remaining anti-MAA monoclonal antibodies displayed various degrees of heterogeneity within individual neoplastic lesions and among different tumors of the same histiotype.

Reactivity of Uveal Melanoma Lesions with Anti-HLA Monoclonal Antibodies. The results of immunohistochemical staining with anti-HLA monoclonal antibodies of different histiotypes are summarized in Table 5. Representative staining patterns are shown in Fig. 1. The following points are noteworthy: (a) the anti HLA Class I MoAb W6/32 and the anti β2-microglobulin MoAb BBM.1 did not stain the spindle A and B lesions but stained all the mixed and epithelioid type lesions. The staining patterns with the two monoclonal antibodies were similar and ranged between variable and homogeneous; (b) in the lesions from patients Bas, Baf, Boh, Mar, and Zuc not only the cell membrane but also the cytoplasm of melanoma cells was stained by MoAb W6/32 and MoAb BBM.1; (c) HLA Class II antigens were detected only in one lesion of the epithelioid type and in one of the mixed type; (d) staining with monoclonal antibodies specific for the individual gene products of the HLA-D region detected only HLA-DR antigens and only in small cell nests in the lesion from patient Mar and both HLA-DR and -DP antigens in the large proportion of the tumor cell population in the lesion from patient Bra; (e) no lesion was stained by the anti HLA-DQ MoAb TU22.
UVEAL MELANOMA, MAA, HLA ANTIGENS

Table 4 Reactivity of surgically removed uveal melanoma lesions with individual monoclonal antibodies to distinct determinants of HMW-MAA and with their pool surgically removed

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* Patient.
† Histotype: SpA, spindle A; SpB, spindle B; M, mixed type; M(A), mixed type with most A type cells; Ep., epithelioid.
‡ Results of immunostaining: −, negative; ±, isolated positive areas; ±, weakly positive; var, staining of variable intensity and/or distribution with negative areas; +, homogeneously positive; ++, strongly homogeneously positive.

Table 5 Reactivity of surgically removed uveal melanoma lesions with anti-HLA monoclonal antibodies

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* Patient.
† Histotype: SpA, spindle A; SpB, spindle B; M, mixed type; M(A), mixed type with most A type cells; Ep., epithelioid.
‡ Results of immunostaining: −, negative; ±, isolated positive areas; ±, weakly positive; var, staining of variable intensity and/or distribution with negative areas; +, homogeneously positive; ++, strongly homogeneously positive.

DISCUSSION

Immunohistochemical staining with monoclonal antibodies to 14 membrane-bound and two cytoplasmic cutaneous MAA has shown that the expression in 12 surgically removed uveal melanoma lesions of the membrane-bound determinants defined by MoAb TP39.1, MoAb TP36.1, MoAb 345.134, MoAb M111, MoAb CL203.4, and MoAb M2590 and of the cytoplasmic determinants defined by the MoAb 465.12 and 2G-10 is similar to that in cutaneous lesions (18, 20, 30, 39–41). On the other hand, the membrane-bound determinants recognized by MoAb 149.53, MoAb 225.28, MoAb 763.74, MoAb B1.1, MoAb 140.240, MoAb ME20.4, and MoAb R24, have a markedly lower expression in uveal than in cutaneous melanoma lesions (16, 17, 42–44). Furthermore, the membrane-bound determinants defined by MoAb 376.96, MoAb ME311, and MoAb ME361 were not detected in the uveal melanoma lesions analyzed. Our results are in agreement with those reported by van der Pol et al. (9) who have analyzed uveal melanoma lesions with MoAb 225.28 and R24 but are at variance with those reported by Bomani et al. (11) who have tested the MoAb 225.28, 763.74, and 376.96. Since the same monoclonal antibodies were used by the various investigators, the conflicting results may reflect differences in the characteristics of the lesions analyzed and/or in the sensitivity of the methods used. The latter possibility is supported by our own finding that incubation with the pool of the anti-HMW-MAA MoAb 149.53, 225.28, and 763.74 has resulted in the specific staining of lesions which were not stained by the individual anti-HMW-MAA monoclonal antibodies. A similar finding has also been obtained with cutaneous melanoma lesions (42).

The expression of HLA antigens in uveal melanoma lesions displays differences and shares features with that in cutaneous melanoma lesions. As in the latter (11, 14), the staining of uveal melanoma lesions may not be restricted to the membrane but may occur also in the cytoplasm of melanoma cells. Furthermore, the lack of expression of HLA Class I and Class II antigens in pure spindle A and B uveal melanoma lesions, which are considered biologically the counterparts of nevocellular nevi (38), parallels similar findings obtained with benign cutaneous lesions of melanocytic origin A (for review, see Ref. 4). Lastly, as in cutaneous melanoma lesions (for review, see Ref. 4), in uveal melanoma lesions the gene products of the HLA-D region have a differential expression, HLA-DR antigens being more frequently expressed than HLA-DQ and -DP antigens. On the other hand, the expression of HLA Class II antigens in about 16% of the uveal melanoma lesions tested is markedly lower than that found in cutaneous melanoma lesions. A recent review of the literature (4) has shown that the overall frequency of expression of HLA Class II antigens in surgically removed primary and metastatic cutaneous and visceral organ melanoma lesions is about 49% with values ranging between a minimum of 0% and a maximum of 100%.

Our results about the high frequency of expression of HLA Class I antigens in uveal melanoma lesions are similar to those reported by Jager et al. (13). However, the latter investigators found no relationship between expression of HLA Class I antigens and histiotype of melanoma lesions. Our results about the frequency of expression of HLA Class II antigens in uveal melanoma lesions cannot be compared to those reported by Jager et al. (13), since they do not indicate the exact percentage of positive lesions.

Besides HLA antigens the determinants recognized by MoAb B1.1, by MoAb CL203.4, and by MoAb R24 display a preferential distribution in lesions of the mixed and epithelioid type. The latter have a worse 5-year survival than pure spindle A and B type lesions (45). These results parallel at least in part findings with cutaneous melanoma lesions. The level of HLA Class II antigens in primary cutaneous melanoma lesions is inversely correlated with the prognosis for the disease (46). Furthermore, the MoAb CL203.4 displays a preferential reactivity with primary lesions with high invasiveness and a poor clinical outcome.4

Since radiolabeled anti HMW-MAA and anti-M, p97,000 MAA monoclonal antibodies have been successfully used to image cutaneous melanoma lesions (47, 48), the reactivity


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clonal antibody would yield a high percentage of false negative epithelial cells of the ciliary body and with a number of normal applications to image ocular melanoma lesions. The homogeneous fluorescence immunoangiography as complementary tests in the results. On the other hand, the positive staining of the majority p97,000 MAA (49) suggests that radioimaging with this monoclonal antibody would yield a high percentage of false negative results. On the other hand, the positive staining of the majority of uveal melanoma lesions tested of MoAb 140.240 which recognizes the Mr 9-0-acetyl-GDα. J. Biol. Chem., 260: 14556-14563, 1985. 


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