Level of HLA Antigens in Locoregional Metastases and Clinical Course of the Disease in Patients with Melanoma

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ABSTRACT

Immunohistochemical staining with monoclonal antibodies showed marked variations in the percentage of melanoma cells stained by anti-HLA Class I and anti-HLA Class II monoclonal antibodies among 48 locoregional metastases removed from 39 patients with malignant melanoma. On the other hand there was limited variation in the percentage of melanoma cells stained by anti-HLA antibodies in autologous locoregional metastases removed from 8 of 9 patients. In the remaining patient marked differences were found in the percentage of melanoma cells stained by anti-HLA Class I antibodies in the two parts of the lymph node metastasis analyzed. Therefore this patient was not included in additional analyses to correlate the level of expression of HLA antigens with the clinical course of the disease.

In all the lesions tested the percentage of melanoma cells stained by anti-HLA Class II antibodies was lower than or equal to but never higher than that stained by anti-HLA Class I antibodies. According to the level of expression of HLA Class I and Class II antigens the 38 patients could be divided into three groups: Pattern A included lesions with more than 50% of tumor cells stained by anti-HLA Class I antibodies (mean, 86.1; median, 85) and 50% or less by anti-HLA Class II antibodies (mean, 10.5; median, 5); Pattern B included lesions with 50% or less tumor cells stained by anti-HLA Class I antibodies (mean, 14.9; median, 5) and by anti-HLA Class II antibodies (mean, 4.1; median, 1); Pattern C included lesions with more than 50% tumor cells stained by anti-HLA Class I antibodies (mean, 88.8; median, 92) and by anti-HLA Class II antibodies (mean, 70.4; median, 70). The survival of 21 patients with Pattern A was significantly longer than that of 13 and 4 patients with Patterns B and C, respectively. No difference in the survival of patients in the latter two groups was found. These results suggest that HLA antigens play a role in the biology of melanoma and that analysis of the level of expression of HLA antigens in locoregional metastases of patients with melanoma may provide clinically useful information.

INTRODUCTION

As in other animal species (1–3), in humans malignant transformation of cells and tumor progression may be associated with quantitative changes in the expression of histocompatibility antigens (for review, see Ref. 4). These findings in conjunction with the potential role of HLA antigens in the interaction of tumor cells with the host’s immune system have stimulated interest in the characterization of histocompatibility antigens expressed by malignant cells. These investigations have been boosted by the development of anti-HLA monoclonal antibodies which have facilitated the analysis of surgically removed tissues with immunohistochemical techniques.

Because of the potential role of immunological events in the pathogenesis and clinical course of melanoma (for review, see Ref. 5), we have focused our investigations on the expression of HLA antigens by melanoma cells. Analysis of surgically removed lesions of melanocytic origin has detected HLA Class II antigens on melanoma cells in all (6–13) but one study (14). On the other hand HLA Class II antigens have not (6–9, 13) or have exceptionally (10, 15) been detected on nevocellular nevi in situ and on normal melanocytes (6, 16, 17), suggesting a relationship between their malignant transformation and appearance of HLA Class II antigens. A role of HLA Class II antigens in the biology of melanoma cells is suggested by their more frequent expression in metastatic than in primary lesions (9, 12, 13, 18) and by the inverse relationship between the level of expression of HLA Class II antigens in primary lesions and the prognosis for the disease (12). HLA Class I antigens and β2-M are expressed by melanoma cells (7, 11, 13, 18) and normal melanocytes (16). The expression of HLA Class I antigens is reduced more frequently in metastatic than in primary lesions (7, 13, 18) suggesting that in melanoma, as in other types of tumors, loss of HLA Class I antigens expression is associated with a greater aggressiveness (19) or metastatic potential (3, 20) of tumor cells.

The prognosis of patients with primary cutaneous melanoma is largely dependent on the tumor thickness, Clark’s level of invasion, mitotic index, microsatellites, subsite, ulceration, and lymphocytic infiltrate (5, 21). In patients with metastases the most important prognostic factors are thickness and ulceration of the primary tumor, total number of metastases, and time interval between the occurrence of primary melanoma and metastasis (5, 22, 23).

Since the expression of HLA Class II antigens in primary lesions is associated with the prognosis of the disease (12, 18), in the present study we have investigated the relationship between the level of expression of HLA Class I and Class II antigens in metastases and the clinical course of the disease. To reduce the effect of variables which are known to influence the clinical course of the disease (24), we have restricted this study to patients with locoregional metastases.

PATIENTS, MATERIALS AND METHODS

Patients. Forty-eight locoregional metastases were excised from 39 consecutive patients with melanoma who had locoregional disease at the time of excision. Sixteen patients had locoregional (sub)cutaneous metastasis (Stage I) and the remaining 23 had locoregional lymph node metastasis (Stage II) (5). The patients were mainly derived from the Department of Surgery, University Hospital, Leiden, The Netherlands, and from the Department of Dermatology, University Hospital, Muenster, Federal Republic of Germany. Only metastases that were excised electively were included in this study. The interval between detection of the primary tumor and first metastasis ranged between 0
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Immunoperoxidase Procedure. At least two different blocks of each metastasis were examined. In addition five specimens of the same lesion from three patients were analyzed to test the reproducibility of the sampling. Cryostat sections 4 μm thick from these blocks were air-dried, fixed in 10% acetic acid for 10 min, and stained with an indirect immunoperoxidase technique as described earlier (7, 13). The sections were counterstained in Mayer’s hematoxylin and mounted with Aquamount (Hopkin and Williams, Chadwell Heath, England). As a control, monoclonal antibodies were replaced by phosphate buffered saline or mouse immunoglobulins. The percentage of stained tumor cells in each section and the staining intensity were estimated independently by three observers. Variations in the percentages of stained cells enumerated by the three investigators were 10% or less. The average percentage was calculated and used for further analysis. Staining intensity was graded as described earlier (13). Only tumor cells that showed 1+, 2+, or 3+ staining were considered positive.

Survival Analysis. Survival curves were calculated using the method of Kaplan and Meier (28). A log rank test was applied to analyze differences between survival curves (29). The relationship between observations was determined, where appropriate, by a χ² test, Fisher’s exact test, or Student’s test.

RESULTS

Immunohistochemical Staining with Anti-HLA Monoclonal Antibodies. The anti-HLA Class I MoAb BRL and CR1 and the anti β₂µ antibody MoAb OLAC yielded similar staining patterns and stained similar percentages of tumor cells in all but one lesion. Similar results were obtained also with the anti-HLA Class I MoAb W6/32 in all but five cases. In the latter ones a weak dull staining with the MoAb W6/32 was found in tumor

Table 1 Relevant clinicopathological data of 39 cases with locoregional melanoma metastases

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<th>Breslow thickness (mm)</th>
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<th>Interval from primary tumor (mo)</th>
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* LN, lymph node; NA, not applicable; DOD, dead of disease; SC, subcutis; S, skin; NK, not known; NED, no evidence of disease; AWD, alive with disease.
areas that were negative with the other anti-HLA Class I monoclonal antibodies in consecutive sections. These lesions have been classified as negative since the intensity of staining is less than 1+. Staining patterns of the melanoma lesions with anti-HLA-DR, DQ, DP MoAb Q5/13 and with anti-HLA-DR MoAb BRL and OKIa1 were comparable. Lesions from Patients 35, 36, 37, 38, and 39 were available for staining with anti-HLA-DR MoAb Q2/70 and with anti-HLA-DQ MoAb Leu-10 and SPV-L3. Positive staining with the latter two antibodies was obtained only in lesions that were stained by anti-HLA-DR antibodies. In these lesions the percentage of tumor cells stained by anti-HLA-DQ antibodies was lower than that stained by anti-HLA-DR antibodies. Because of the concordance in the staining patterns obtained with the antibodies tested, the results will be presented in terms of expression of HLA Class I antigens and HLA Class II antigens rather than in terms of reactivity of individual antibodies.

The staining of melanoma cells with anti-HLA Class I and Class II monoclonal antibodies displayed both a diffuse intra-cytoplasmic and a peripheral pattern. Tumor cells stained by anti-HLA Class II antibodies were often localized perivascularly or at the edges of tumor cell nests. Tumor cells stained by anti-HLA Class I antibodies displayed a similar distribution in metastases with low HLA Class I antigen expression and a diffuse one in those with high HLA Class I antigen expression. The latter antigens were detected less frequently on small melanoma cells than on larger epithelioid cells. The percentage of tumor cells stained by anti-HLA Class I and by anti-HLA Class II monoclonal antibodies and the staining intensity in the 48 lesions tested are shown in Table 2. Anti-HLA Class I antibodies stained 20% or less tumor cells in 10 metastases, between 21 and 50% in 6 metastases, between 51 and 80% in 11 metastases, and between 81 and 100% in 20 metastases. The two specimens of the metastasis removed from Patient 3 showed marked differences in the percentage of melanoma cells stained by anti-HLA Class I monoclonal antibodies. Anti-HLA Class II antibodies stained 5% tumor cells or more in the large majority of lesions (i.e., 30 of 48 lesions).

The staining intensity was heterogeneous among individual cells within a metastasis. However, the percentages of melanoma cells stained by anti-HLA Class I and anti-HLA Class II monoclonal antibodies were similar among five specimens of the same lesion in the three patients tested. Only the metastasis from Patient 3 showed a marked difference in the percentage of melanoma cells stained by anti-HLA Class I monoclonal antibodies between two different parts of the lesion. Therefore Patient 3 was not included in any analysis. Furthermore limited variation in the percentage of melanoma cells stained by anti-HLA monoclonal antibodies was found in autologous locoregional metastases removed from eight patients (i.e., patients 4, 8, 11, 12, 13, 17, 26, and 34) from whom more than one lesion was available for immunohistochemistry (Table 2). In no patient did such a variation cause a change in the assignment of the lesions to the three groups of HLA patterns which are described in the next section.

Staining Patterns with Anti-HLA Monoclonal Antibodies. Based on the U-shaped frequency distribution of the percentage of melanoma cells stained by anti-HLA Class I antibodies in the metastases (Table 2) two patterns could be discerned: one included lesions with more than 50% tumor cells stained; and the other lesions had 50% or less tumor cells stained. In every lesion the percentage of tumor cells stained by anti-HLA Class I antibodies was the same or higher than that stained by anti-HLA Class II antibodies. The 50% breakpoint was also chosen to classify the metastases for HLA Class II antigens.
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Fig. 1. Immunoperoxidase staining with anti-HLA Class I (A) and anti-HLA Class II (B) monoclonal antibodies of consecutive frozen sections of a lymph node metastasis with HLA expression Pattern A. Melanoma cells are diffusely stained by anti-HLA Class I monoclonal antibodies but are not stained by anti-HLA Class II monoclonal antibodies. Lymphocytes (L) and blood vessels (arrows) are stained by anti-HLA Class I and anti-HLA Class II monoclonal antibodies. × 160, with hematoxylin counterstaining.

median, 1] [low HLA Class II antigen expression] [Fig. 2], was found in 13 patients, with 5 in Stage I and 8 in Stage II; Pattern C, more than 50% tumor cells stained by anti-HLA Class I monoclonal antibodies [mean, 88.8%; median, 92] [high HLA Class I antigen expression] and by anti-HLA Class II monoclonal antibodies [mean, 70.0; median, 70] [high HLA Class II antigen expression] [Fig. 3], was found in 4 patients, all in Stage II. The fourth theoretically possible pattern, i.e., low HLA Class I and high HLA Class II antigen expression, was not found in any patient. The distribution of HLA Class I and Class II antigens in melanoma cells did not differ in the lesions from the patients in the three groups. The distribution of lesions with the different staining patterns was not significantly different (P = 0.2) between patients in Stage I and Stage II.

Relationship between HLA Antigen Expression in Metastases and Survival of Patients with Melanoma. The percentage of melanoma cells stained by anti-HLA Class I and anti-HLA Class II monoclonal antibodies in metastases was correlated with the survival of 38 patients with melanoma. The latter were considered as a whole, since the survival of 16 patients with locoregional cutaneous and s.c. lesions was not significantly different (P = 0.5) from that of 22 patients with lymph node metastases. Survival was measured either (a) from the month of excision of the locoregional metastasis tested or (b) from the month of diagnosis of the first locoregional metastasis to death or last follow-up. Survival curves were calculated separately for a group of 23 patients whose first metastasis was tested with anti-HLA monoclonal antibodies and for a group of 15 patients whose second or later metastasis was tested. The survival of 21 patients whose metastases expressed Pattern A was significantly longer (P = 0.02) than that of the remaining 17 patients whose metastases expressed Pattern B or C [Fig. 4, left]. Separate survival curves of patients with Patterns B and C were also significantly different (P = 0.005) from those of patients with Pattern A but were not different from each other (P > 0.05) [Fig. 4, left]. Statistically significant differences (P = 0.001 and P = 0.003, respectively) were also found when survival was calculated from the time of diagnosis of the first metastasis (Fig. 5, right). The relationship between survival and level of HLA antigen expression is shown in Fig. 6. The distribution of patients in 3 clusters of low and high level of expression of HLA antigens is in agreement with Patterns A, B, and C.

Patients with a primary tumor with a thickness of 1.5 mm or less tended to have Pattern A more frequently than patients with thicker primary lesions. However, the correlation between the level of HLA antigens and the Breslow thickness of the
Ig was stained by anti-HLA Class I monoclonal antibodies in all patients with melanoma has shown that at least 1% of melanoma cells under systematic chemotherapy before excision of the metastasis. It is also noteworthy that all patients investigated had not been treated with previous therapy. Combination therapy for widespread malignant melanoma (5) it is of note that there is no consistently effective single agent or combination of agents.

Similarly, the interval between primary melanoma and metastasis (P = 0.5) and number of metastasis (P = 0.7) (22, 23, 30). Furthermore, although there is no consistently effective single agent or combination therapy for widespread malignant melanoma (5) it is also noteworthy that all patients investigated had not been under systematic chemotherapy before excision of the metastasis analyzed.

**DISCUSSION**

Immunohistochemical staining with a panel of anti-HLA monoclonal antibodies of locoregional metastases from patients with melanoma has shown that at least 1% of melanoma cells was stained by anti-HLA Class I monoclonal antibodies in all 48 lesions tested and by anti-HLA Class II monoclonal antibodies in 42 of the 48 lesions tested. In agreement with a previous study (13), monoclonal antibodies recognizing distinct monomorphic determinants of HLA Class II antigens yielded similar staining patterns. A similar conclusion was reached with monoclonal antibodies to distinct monomorphic determinants of HLA Class I antigens for all but one lesion; in the lesion from Patient 2 the anti-β2-μ monoclonal antibody stained a lower percentage of melanoma cells than monoclonal antibodies to determinants of the heavy chain of HLA Class I antigens. This patient was included among those with low expression of HLA Class I antigens, since lack of expression of β2-μ might be associated with functional abnormalities of HLA Class I antigens. The proportion of lesions that expressed HLA Class I antigens by 50% or less melanoma cells in our study was higher than that reported recently by Taramelli et al. (31) (34% versus 15%, respectively). The latter investigators analyzed with a cytofluorograph melanoma cells stained with anti-HLA monoclonal antibodies following isolation from surgically removed lesions. The discrepancy between the results of Taramelli et al. and our own may reflect differences in the sensitivity of the assay system used in the two investigations and/or selection of melanoma cell subpopulations during the preparation of the cell suspension. In agreement with the results of Taramelli et al. (31) and with our previous results (32–34) the percentage of melanoma cells stained by anti-HLA-DQ monoclonal antibodies was lower than or similar to that stained by anti-HLA-DR monoclonal antibodies.

The percentage of melanoma cells stained by anti-HLA monoclonal antibodies varied markedly among the metastases from the patients investigated, as has been reported previously (9, 11, 13, 18, 35). On the other hand a much less marked variation was found among autologous locoregional metastases of individual patients. The latter finding is at variance with the previously described high degree of heterogeneity in the expression of HLA antigens among distant autologous metastases (11, 18). Melanoma cells stained by anti-HLA Class I monoclonal antibodies displayed a diffuse distribution in lesions with high HLA Class I antigen expression and a perivascular one in lesions with low HLA Class I antigen expression. In agreement with previous results (13, 35) melanoma cells stained by anti-HLA Class II monoclonal antibodies were found in perivascular areas. It remains to be determined whether the expression of HLA antigens by melanoma cells is modulated by a factor produced by perivascular, vascular, and/or intravascular cells.

Previous studies have shown a relationship between the level of HLA Class I and HLA Class II antigen expression in primary cutaneous melanoma lesions and the degree of lymphocytic peritumoral (7) and intratumoral (12) infiltrate, respectively. In the present study only (sub)cutaneous metastases could be tested for lymphocyte infiltrates, since infiltrating lymphocytes cannot be discriminated from preexisting lymphocytes in lymph node metastases. The results we have obtained with the limited number of lesions analyzed in the present investigation suggest that perilesional lymphocytic infiltrates are scarce or absent in pattern B lesions and that a marked intralesional lymphocytic infiltrate is found especially in Pattern C lesions. These findings have been recently confirmed by analyzing an additional set of locoregional metastases and are in agreement with the results obtained with primary cutaneous melanoma lesions (12, 18).

The present investigation has shown for the first time a
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statistically significant association between the level of expression of HLA antigens in melanoma metastases and the clinical course of the disease. The potential clinical significance of our findings is stressed by the lack of correlation between level of HLA antigens in metastases and known prognostic parameters for patients in Stages I or II, such as the number of locoregional metastases and the interval between primary melanoma and metastasis (22, 23, 30). Only Breslow thickness exerted an influence on the prognostic value of HLA antigen expression patterns. If additional studies on a large number of patients confirm our findings, then quantitation of HLA antigens in metastatic lesions may become a useful parameter in the clinical evaluation of patients with melanoma.

Previous studies (11, 18, 32, 36, 37) have analyzed melanoma metastases not only with anti-HLA monoclonal antibodies but also with monoclonal antibodies to human MAA. The latter included melanocytic differentiation markers, such as HMW-MAA (11), and markers of tumor progression, such as gp130, p76, gp75, gp89, and gp113 (37). In these studies the level of expression of MAA in melanoma metastases showed no obvious relationship with that of HLA antigens and with the clinical course of the disease. Because of the lack of tissue in the present investigation we could test only 19 melanoma metastases with anti-HMW-MAA and anti-p97 MAA monoclonal antibodies. The percentage of melanoma cells stained by anti-HLA Class I monoclonal antibodies was significantly correlated with that stained by anti-HMW-MAA (P = 0.002) and anti-p97 MAA (P = 0.02) monoclonal antibodies. A much larger number of melanoma lesions must be tested to assess the validity and the significance of the association between the expression of HLA antigens and MAA we have found.

The mechanism(s) underlying the association between level of HLA antigens in metastases and clinical course of the disease in patients with melanoma are only speculative at this time. The poor prognosis of patients with a low expression of HLA Class I antigens in metastases may reflect the inability of cytotoxic T-cells to recognize and kill melanoma cells, in view of the role of these antigens in the interaction between cytotoxic T-cells and target cells (38). Such a mechanism has been demonstrated in a highly oncogenic adenovirus transformed rat tumor cell line with low expression of Class I histocompatibility antigens which was not susceptible to T-cell killing (39). The oncogenicity of this cell line was drastically reduced following transfection with a gene encoding Class I histocompatibility antigens (40). Similar results have been obtained in two murine tumor systems (41, 42).

The poor prognosis of a small group of patients with a high expression of HLA Class I and HLA Class II antigens (Pattern C) in their locoregional metastases is a surprising but not completely unexpected result. Previous studies have shown that the level of HLA Class II antigens is higher in primary melanoma tumors thicker than 1.5 mm than in thinner tumors (12), is high in primary lesions that give rise to early metastases (12), and is more frequent in metastatic lesions than in primary lesions (9, 11, 13, 18). Furthermore HLA Class II antigens bearing melanoma cells from metastatic lesions are not able to induce proliferation of autologous T-cells (43, 44) and display suppressive activity. The latter correlates with the level of expression of HLA Class II antigens (45). Alternatively HLA Class II antigen expression by melanoma cells has been suggested to represent an early stage of differentiation (17) that may be associated with enhanced proliferative and metastatic capacities. Lastly, HLA Class II antigen expression may be the result of malignant transformation, as suggested by the appearance of HLA Class II antigens on melanocytes transformed with retroviral ras oncogenes (46).

The association between level of expression of HLA Class I and Class II antigens in metastases and prognosis might not be restricted to patients with melanoma with locoregional metastases. Low expression of HLA Class I antigens or high expression of HLA Class II antigens has been reported in the large majority of metastases from seven patients with Stage IV melanoma (11) and in the more malignant types of ovarian tumors (47).

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REFERENCES


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