HLA expression in a primary uveal melanoma, its cell line, and four of its metastases


Abstract

Background—The level of HLA expression on a tumour may influence the immunological response against this tumour, and vice versa. HLA expression was determined in a primary uveal melanoma, its metastases, and on a cell line derived from this melanoma, and the presence and type of infiltrate in tissue sections were also studied.

Methods—Immunohistochemistry with monoclonal antibodies (MAbs) against HLA class I and II, T cells, NK cells, and macrophages.

Results—Primary and metastatic lesions, as well as the cell line showed high levels of expression of the monomorphic determinants of HLA class I. Expression of the polymorphic HLA-A2 and HLA-A3 antigens was decreased on metastases to the skin and liver. HLA-Bw4 expression was low on all lesions, as well as expression of HLA class II. Tumour infiltrating cells consisted mainly of CD3, CD4, and CD8 positive cells. Expression on the cell line corresponded to expression on the primary tumour.

Conclusion—The primary uveal melanoma as well as the cell line showed a high expression of monomorphic and polymorphic HLA-A antigens, while metastases showed a high expression of monomorphic and a lower expression of polymorphic antigens. This variation in expression may support tumour cell escape from NK cells as well as CTL mediated lysis.

Changes in HLA expression often occur during carcinogenesis. Since the function of cytotoxic T lymphocytes (CTLs), as well as of natural killer (NK) cells, is directly influenced by expression of HLA class I antigens, changes in HLA class I expression might influence an antitumour immune response, and, therefore, prognosis. An association between HLA class I expression and prognosis was observed, for instance, in larynx and breast carcinomas: a lack of HLA class I expression correlated with a more aggressive tumour behaviour and a worse prognosis. In skin melanoma, the transformation of normal skin melanocytes to melanoma cells is frequently accompanied by a decreased expression of HLA class I antigens, and complete or selective loss of expression of HLA class I has been observed more frequently in metastatic compared with primary skin melanomas. Van Duinen et al showed an association between lack of HLA class I expression in locoregional metastases and poor prognosis in patients with stage II cutaneous melanoma.

Although several authors have studied the expression of monomorphic and polymorphic HLA class I and/or II expression in primary uveal melanomas, data on HLA expression on metastases of uveal melanoma are still scarce. To our knowledge, only one such study has been performed, in which HLA class I expression was studied on a culture derived from a primary uveal melanoma and on two separate cell cultures derived from liver metastases of the same patient. In Tran et al’s report, it was concluded that both primary and metastatic tumour cells expressed HLA class I.

In the present study, we determined the level of expression of HLA class I and II molecules on a primary intraocular melanoma, on four of its metastases, and on a cell line obtained from the primary tumour. The presence of tumour infiltrating cells in these lesions was also assessed, and compared with the expression of the HLA antigens.

Materials and methods

CLINICAL AND HISTOPATHOLOGICAL FINDINGS

The patient was a 76-year-old woman referred to the department of ophthalmology of Leiden University Hospital with a large tumour in the right orbit extending from the eye (largest tumour diameter 20 mm). On examination, the visual acuity of the right eye was 0 and the best corrected visual acuity of the left eye was 0.3. The right eyeball had been displaced superotemporally by a large tumour, and vessel growth was observed throughout the eyeball. With the exception of a cortical cataract, the left eye showed no abnormalities. Computed tomography of the right orbit revealed a small and deformed eyeball with tumour outgrowth into orbital structures. Size and location of the malignancy excluded conservative treatment, and orbital exenteration was performed. Before orbital exenteration, the patient did not receive any treatment.

Following exenteration, the tumour was dissected. One part of the tumour was prepared for tissue culture, and has given rise to the establishment of a well characterised cell line. Two different parts of the tumour from randomly chosen areas were snap frozen and stored at −80°C until sectioning for immuno-
Intensity of staining: + = slight, ++ = moderate, and +++ = strong staining.

Score: 1 = less than 5% of cells staining positive; 2 = 5–25%; 3 = 26–50%; 4 = 51–75%; 5 = 76–100%.

*Melanoma associated antigen, as determined with MAb NKI-beteb.

Table 1: Quantitative counts of the percentage of cells in sections and cytospots of primary uveal melanoma, and in sections of metastatised uveal melanoma reacting with monoclonal antibodies directed against HLA class I antigens.

<table>
<thead>
<tr>
<th>Antigen/MAb</th>
<th>Cell line</th>
<th>Primary tumour</th>
<th>Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part 1</td>
<td>Part 2</td>
<td>Adrenal gland</td>
<td>Skin</td>
</tr>
<tr>
<td>MelAss Ag*</td>
<td>NKI-beteb</td>
<td>5/+</td>
<td>5/+</td>
</tr>
<tr>
<td>HLA-A,B,C</td>
<td>W6/32</td>
<td>5/+</td>
<td>5/+</td>
</tr>
<tr>
<td>b2-microglobulin</td>
<td>BBM1</td>
<td>5/+</td>
<td>5/+</td>
</tr>
<tr>
<td>A locus</td>
<td>HCA2</td>
<td>5/+</td>
<td>5/+</td>
</tr>
<tr>
<td>A2</td>
<td>MA2.1</td>
<td>5/+</td>
<td>5/+</td>
</tr>
<tr>
<td>A3</td>
<td>GAP-A3</td>
<td>5/+</td>
<td>5/+</td>
</tr>
<tr>
<td>B locus</td>
<td>HC10</td>
<td>5/+</td>
<td>5/+</td>
</tr>
<tr>
<td>Bw4</td>
<td>116 5 28</td>
<td>3/+</td>
<td>1/+</td>
</tr>
<tr>
<td>DP</td>
<td>B7/21.2</td>
<td>0</td>
<td>2/+</td>
</tr>
<tr>
<td>DQ</td>
<td>SPV-4.3</td>
<td>0</td>
<td>2/+</td>
</tr>
<tr>
<td>DR</td>
<td>B811.2</td>
<td>0</td>
<td>2/+</td>
</tr>
</tbody>
</table>

*Melanoma associated antigen, as determined with MAb NKI-beteb.

Intensity of staining: + = slight, ++ = moderate, and +++ = strong staining.

Histology. The remainder of the tumour was processed for histopathological examination by an ocular pathologist (D De W-R) (paraffin embedded tissue sections stained with haematoxylin and eosin). Histopathology and immunohistochemistry with the melanoma antigen specific MAb NKI-beteb (Monosan, Uden, Netherlands) revealed a malignant melanoma with extensive extrabulbar growth. The tumour was histologically classified as an epitheloid uveal melanoma. Mitoses were counted in 15 high power fields (HPF) with a total magnification of ×320, using an eyepiece grid (15 HPF=4.3 mm²). Two and a half year after enucleation, the patient died as a result of uveal melanoma metastases. Obduction showed multiple melanoma metastases in various sites. Metastatic lesions were dissected from the liver, adrenal gland, the skin, and the heart, and snap frozen and stored at –80°C.

HLA TYPING
DNA analysis on peripheral blood leucocytes revealed that the patient had the following HLA type: HLA class I: A*02, A*03, A*0206, B*12, B*44, B*05, B*04, C*0501, C*14; HLA class II: DR4, DR53, DQ7, DQ8, DQ3.

IMMUNOHISTOCHEMISTRY
For immunohistochemical staining, anti-HLA monoclonal antibodies were used in agreement with the HLA type of the patient (Table 1). References of the anti-HLA MAbs have been described previously. For infiltrate analysis, the number of positively stained tumour cells were estimated and expressed as the percentage of the total number of tumour cells in the analysed section. The numbers of positively stained tumour cells were evaluated without access to histological data.

ANALYSIS OF ANTIGEN EXPRESSION
For each antibody, the numbers of positively stained tumour cells were estimated and expressed as the percentage of the total number of tumour cells in the analysed section. Percentages were put into five categories (1 = <5%, 2 = 5–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–100%). The slides were examined independently by two observers. Interobserver disagreements did not exceed one class, and in case of interobserver disagreement, consensus could be reached during joint evaluation.

Results
Standard histopathology showed that the metastases had the same cell type (that is, epitheloid) as the primary uveal melanoma. The tumour tissue of the metastases showed less coherence than the primary tumour, and little variation in vessel pattern. There were fewer normal and more atypical mitoses in metastases in comparison with the primary tumour (0–5/15 HPF, and 35/15 HPF, respectively).

Immunohistochemical staining was performed with several anti-HLA class I and II MAbs on a primary uveal melanoma and four metastases. In primary as well as metastatic lesions, vessels stained positive with all anti-HLA MAbs tested (Table 1). In all tissue sections studied—that is, of the primary tumour as well as of the metastatic lesions, more than 75% of the tumour cells stained positively with the MAb directed against the melanoma associated antigen NKI-beteb, the anti-HLA class I MAb W6/32, and the anti-β2 microglobulin.
microglobulin MAb BBM1. The MAbs recognizing polymorphic antigens showed a more variable pattern. The two different parts obtained from the primary tumour stained similarly, with exception of the anti-A and B locus specific MAbs. One of the parts showed a slightly lower expression with these MAbs. Some of the metastases stained only partly...
positive with the three anti-HLA-A MAbs. Metastases in skin and liver showed a lower expression of HLA-A2 and HLA-A3 than the primary melanoma and the other two metastases (Fig 1C, 1E, and 1F).

With regard to HLA-B, variable percentages and intensities of staining were found on primary tumour parts as well as on the metastases. The percentage of tumour cells staining positively for HLA-Bw4 did not exceed 25% in the primary and the metastatic lesions.

For HLA class II, a similarity in staining was observed between primary tumour parts and metastases (Fig 1D, 1G, and 1H), with more than 5% of the tumour cells staining positively. NK1-beteb positive areas were selected for evaluation. In addition, scattered HLA class II positive non-tumour cells were observed in all sections.

The results of infiltrate analysis with immunohistochemistry are shown in Table 2. In the primary tumour, the CD3+ and CD4+ cells accounted for the majority of tumour infiltrating cells. High densities of CD3+ and CD4+ cells were especially found in skin and liver metastases. CD8+ cells were also observed, although in smaller numbers. No tumour infiltrating CD68+ macrophages or CD56+ NK cells were observed in the two parts of the primary tumour. In the metastatic lesions, a few CD56+ NK cells were observed in small numbers in the liver metastasis. Some CD68+ macrophages were observed in the liver and skin lesions. However, CD68 does not recognise all macrophages, but we used this marker to make a comparison of different sites. In all metastatic lesions, T cells were more frequently observed than NK cells or macrophages.

### IMMUNOCYTOCHEMISTRY

We determined HLA expression on cytospin preparations of cell line 92-1, established from the primary tumour of this patient,13 using the same anti-HLA MAbs as for the tissue sections. Expression levels for all anti-HLA class I antigens except Bw4 were similar to those on the primary tumour parts; the percentage of cells staining for Bw4 was higher on the cytospot, although with a lower intensity of staining (Table 1). No expression of HLA class II antigens was detected on the cell line.

### Discussion

We compared the expression of HLA class I and II antigens on cells of a primary uveal melanoma, on four of its metastases, and on a cell line obtained from the primary tumour. A high expression of HLA class I was observed on all lesions as well as on the cell line, while expression of HLA class II was low or absent.
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could not account for the HLA class II staining. In previous studies, other authors also reported only small numbers of infiltrating cells in uveal melanoma. Remarkably, cell line 92-1 did not demonstrate any HLA class II expression. The observation that a proportion of melanoma cells are HLA-DR positive is not surprising, since in our laboratory Jager et al. and De Waard-Siebinga et al. have previously demonstrated that sections of human uveal melanoma express HLA class II antigens.

A possible explanation for the expression of HLA class II on human uveal melanoma sections, is the influence of (cytokines produced by) tumour infiltrating cells on antigen expression. This is further supported by the finding of De Waard-Siebinga, who observed that uveal melanoma cells grown in vitro lost expression of the class II determinant HLA-DR, while expression of HLA class I on cultured melanoma cells remained similar to the expression on the original tumour. In conclusion, our present data are in line with previous studies that support the following theory: because of their mainly haematogenous route of metastasis, uveal melanoma cells with a high expression of monomorphic HLA class I have a better chance to evade NK cell mediated lysis and, hence, are well suited to give rise to metastases. In the tissues, CTLs may attack the melanoma cells, as long as they show a good expression of HLA class I. Immunotherapy may specifically be aimed against these metastases.

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