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Ia-like Antigen Expression on Biologically Different Human Melanoma Cell Lines*

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Abstract—*Ia-like antigen binding of a large panel of monoclonal antibodies (six anti-human Ia-like monoclonal antibodies and ten murine anti-Ia monoclonal antibodies cross-reactive with human Ia-like antigens) were compared on seven permanent human melanoma cell lines by radioimmunoassay. Cell lines were initiated from primary or metastatic tumors and presented various levels of tumorigenicity (assessed by heterotransplantation in nude mice) and pigmentation (shown by 5-S-cysteinyldopa determination and cytological data). Two cell lines originated from the same primary melanoma, while two other pairs of cell lines originated from superficial spreading melanoma or metastatic lymph node of the same patients. Identical Ia-like allodeterminants were found in cell lines of the same individual origin. Quantitative expression of β_2-microglobulin and Ia-like antigens was similar in all cell lines except for one, in which these molecules were expressed in lower amounts. These results indicate that Ia-like antigen expression of the cell lines is unrelated to primary or metastatic origin, degree of pigmentation and ability to grow in nude mice.*

INTRODUCTION

ABSENT in normal melanocytes [1, 2], Ia-like antigens with genetically determined allospecificity [3] appear to be expressed with different intensity in a variable percentage of human malignant melanomas [2, 4–7]. A likely possibility is that the heteregeneous pattern of Ia-like antigen expression may reflect differences in the biological properties of malignant melanocytes. Since Ia-like antigens seem to be involved in the regulation of cell proliferation and immune recognition processes [8, 9], and since these molecules are present in various human tumors [3, 10, 11], the detection of Ia-like antigens in melanomas might depend on their degree of malignancy. Numerous differences in respect to their ability to escape host immune destruction and to colonize distant sites also exist between primary and metastatic tumors and within these two tumor categories. Furthermore, the selective occurrence of Ia-like antigens depends upon the differentiation level in normal and malignant cells of the hematopoietic and lymphoid systems [12–14]. In analogy to these findings, it can be considered that Ia-like antigens may be differentiation antigens in melanomas [1, 2, 4, 15].

The phenotypic expression of Ia-like antigens was studied in seven established human melanoma cell lines which exhibited differences in tumor origin, degree of tumorigenicity and pigmentation level. Some of the cell lines were derived from the same tumor or tumors from the same patients. Comparison of Ia-like antigen expression between the cell lines was carried out by means of radioimmunoassay, using a battery of anti-human Ia-like monoclonal antibodies and murine anti-Ia monoclonal alloantibodies which cross-reacted with human Ia-like antigens.

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MATERIALS AND METHODS

Cell lines

Seven permanent cell lines originating from human malignant melanomas from four patients were used (Table 1). An human embryonic lung fibroblast, MRC 5, was included as negative

control for Ia-like antigen expression. The cell lines were cultured as previously described [16] in minimum essential medium supplemented with 15% fetal calf serum (FCS) and antibiotics (MEM-FCS). The same batch of FCS (No. 101, 163, Seromed, Biopro, France) was used to avoid short-term variations in antigenic expression [17]. The results reported here were performed on exponential phase cell monolayers of a limited number of subcultures to minimize long-term cell changes. The study of morphology at the optical and ultrastructural levels, the determination of 5-S-CD by high-pressure liquid chromatography and the tumorigenic assay by heteretransplantation in nude mice have been previously described [16] and the results are shown in Table 2.

Neuraminidase treatment

Cell monolayers were washed three times with phosphate-buffered saline (PBS), pH 5.5, incubated with 10 U/ml of *Vibrio cholerae* neuraminidase (Behringwerke, West Germany) in PBS, pH 5.5, for 1 hr at 37°C and then washed with PBS, pH 7.4.

Monoclonal antibodies (mAbs)

Anti-human Ia-like mAbs, L1-1.6, V1-15.C and 62-3.34 [18], were ascitic fluids and three others, B8-11.2, B8 12.2 and B8-13.6 [19], were purified antibodies (Table 3). Murine anti-Ia-mAbs [20, 21], cross-reactive with human Ia-like antigens, were derived from fusion between BALB/c myeloma (either NS1-Ag 4.1 or X63-Ag

Table 1. Origin of human melanoma cell lines

Established cell lines	Patients* Sex	Age (yr)	Site of tumor	Tumor diagnosis*	Range of subcultures studied‡
			Primary tumors		
IGR 3	♂	60	arm	NM (IV)§	35–45 (37)
IGR 4	♂	60	arm	NM (II)	83–97 (85)
IGR 39	♂	26	leg	SSM (IV)	17–32 (21)
IPC 164	♀	44	leg	SSM (IV)	32–45 (41)
			Metastatic tumors		
IGR 2	♀	55	axilla	LN	86–104 (102)
IGR 37	♂	26	groin	LN	15–35 (16)
IPC 167	♀	44	groin	LN	19–33 (21)

*IGR 3 and IGR 4 lines were derived from the same primary melanoma of an untreated patient. IGR 39 and IGR 37 lines were initiated from simultaneously excised tumors of the same untreated patient. IPC 164 line was initiated from a primary tumour of a patient treated with DTIC, CCNU and vinblastine, whereas IPC 167 line was originated from a metastatic melanoma which appeared in the same patient 3 months after primary tumor excision. IGR 2 line was derived from a metastatic melanoma of an untreated patient whose primary tumor had been excised 2 months previously.

NM = nodular melanoma; SSM = superficial spreading melanoma; LN = lymph node.

‡Tumorigenicity of cell lines was assayed on subculture shown in parentheses.

§Level of invasion according to Clark's classification (1969).

Table 2. Pigmentation level and tumorigenicity of human melanoma cell lines

Cell lines	Pigmentation‡ (melanosomes*)	5-S-CD: in medium (ng/ml)	5-S-CD: in cells (ng/1 × 10^6)	Heterotransplantation: No. of tumors/No. of grafted mice: 1 × 10^6 cells	2 × 10^6 cells	5 × 10^6 cells	1 × 10^7 cells	Size of tumor†	Pigmentation of tumor‡
				Cell lines derived from primary melanomas					
IGR 3	A (f + g)	4.0	0	0/2	1/4	2/2		0.5	A
IGR 4	A (g)	4.0	0	2/2	2/2			6.0	A
IGR 39	A (g)	5.0	0	2/5		2/2	1/1	0.5	A
IPC 164	P (g)	92.0	27.0	1/2	1/1	1/1		1.5	A + P
				Cell lines derived from metastatic melanomas					
IGR 2	P (f)	186.0	48.0	5/7	2/2			6.0	P
IGR 37	P (f)	48.1	13.1	2/2	2/2			3.5	A + P
IPC 167	A (f + g)	0	0	0/2	1/2	1/1		0.3	A

*f = fibrillar melanosomes; g = granular melanosomes.

†Nude mice received subcutaneous dorsal injections of 1 × 10^6 cells.

‡A = achromic, P = pigmented.

8.653) and spleen cells from A. TH ($K^sI^sD^d$) mice immune to lymphoid cells from A.T.L. ($K^sI^kD^d$) mice (Table 4) and were used as purified antibodies. Recent results indicated that mAb H39-49.5 recognized a monomorphic determinant in human DR-antigens [22], whereas mAb H40-315.7 appeared to recognize an epitope present in a broad family of human Ia-like molecules with DC1 characteristics in the α chain and a more heterogeneous β chain [23]. Positive control was a purified mAb, B1.1G6 [20, 21], against human β2-microglobulin (β2m), which is considered to be antigenetically invariant. A purified anti-Ia^k mAb, H8-109-13 [19, 20], which did not cross-react with Ia-like antigens was used as negative control to rule out weak positive reactions due to the possible presence of Fc receptors on melanocytes.

Cell surface radioimmunoassay

Cell monolayer radioimmunoassay [24] was slightly modified. Cells (5×10^4) in MEM-FCS were seeded in wells of Terasaki microtest plates. Two to four days after, subconfluent cell monolayers were washed three times with MEM-FCS and incubated with mAb (usually 15 μg) for 1 hr at 37°C. After washing as above, ^{125}I-labeled $(Fab')_2$ of goat anti-murine IgG (1 μg; 50,000 counts/min/μg) was added and incubated for 2 hr at 4°C. Cells were then washed and lysed with 1% sodium dodecylsulfate. The bound radioactivity was determined by counting in a Gamma counter.

Table 3. Characteristics of monoclonal anti-human Ia-like antibodies [18, 19]

Designation of hybrid clone	Parental lymphoid cells	Parental myeloma	Ig class*	Ia-like specificity†
L1-1.6	BALB/c mouse anti-LKT (lymphoblastoid cell line) splenocytes	NS1-Ag 4.1	Ig M	non-polymorphic
V1-15.C	BALB/c mouse anti-T51 (lymphoblastoid cell line) splenocytes	SP_2O	IgG 2b	non-polymorphic
62-3.34	Lewis rat anti-RAJI (Burkitt cell line) splenocytes	X63-Ag8.653	IgG 2b	non-polymorphic
B8-11.2	BALB/c mouse anti-human cytotoxic T lymphocytes	NS1-Ag 4.1	IgG 2b	non-polymorphic
B8-12.2	BALB/c mouse anti-human cytotoxic T lymphocytes	NS1-Ag 4.1	IgG 2b	non-polymorphic
B8-13.6	BALB/c mouse anti-human cytotoxic T lymphocytes	NS1-Ag 4.1	IgG 2b	non-polymorphic

*Determined by Outcherlony gel immunodiffusion.
†Tested by cytotoxicity and/or binding assay on panel of human B lymphocytes.

Table 4. Characteristics of monoclonal anti-Ia^k antibodies [20, 21]

Hybridomas	Ig class*	H-2 specificity†	Cross-reactions with human B lymphocytes‡
H8-15.9	IgG2a	I-A	polymorphic broad
H39-49.5	,,	I-A	non-polymorphic
H40-481.3	,,	I-A	NT§
H40-164.3	,,	I-A + I-E	polymorphic broad
H10-81.10	,,	I-E	polymorphic broad
H39-91.1	,,	I-E	polymorphic broad
H40-242.3	,,	I-E	non-polymorphic
H40-281-2	,,	I-E	NT
H40-315.7	,,	I-E	polymorphic broad
H40-394.2	,,	I-E	polymorphic narrow

*Determined by Outcherlony's immunodiffusion.
†Established by competitive assays on spleen cells from inbred strains of mice with recombinant or standard H-2 haplotypes.
‡Performed against human B lymphocytes from two different panels of healthy blood donors.
§NT = not tested.

The amounts of mAb and radiolabeled probe were in large excess. The percentage of specific binding of each mAb was calculated as:

$$\% = \frac{A - B}{C - B} \times 100,$$

where values are bound counts/min per well for cells incubated respectively with reactive anti-mAb (*A*), irrelevant mAb (H8-109.13) (*B*) and positive control (B1-IG6) (*C*). The ratio of binding values for both controls was usually ≥ 5. The values presented were the mean of at least three separate experiments, each determination performed in triplicate.

Quantitative absorption of anti-β2m mAb

Cells were removed from the tissue culture flask by treatment with 0.5 mM EDTA in PBS and washed extensively with MEM. Cell pellets corresponding to equivalent amounts of protein (2-fold dilutions ranging from 8 to 0.125 mg protein) were mixed with B1.1G6 (100 μl diluted 1/250, yielding 100% fixation in radioimmunoassay) and incubated for 1 hr, first at room temperature and then at 4°C. After removal of absorbing cells by centrifugation, the absorbed antibodies were finally titrated in parallel with unabsorbed diluted B1.1G6 for residual activity in radioimmunoassay using IGR 37 cell line as target cells.

RESULTS

Comparative reactivity of anti-human Ia-like mAbs

Under the standardized culture conditions used, it appeared that the extent to which cell lines bound mAbs differed only slightly over several months. The results obtained with cell-surface binding assay are summarized in Fig. 1. In contrast to the cell line MRC 5, which was completely negative as expected, the seven melanoma cell lines gave a complex pattern of positive and negative reactions depending on the cell line and the mAb. The spectrum of anti-Ia-like mAb binding was qualitatively identical in pairs of cell lines with the same individual origin. Absorption of anti-β2m mAb showed that the amount of β2m was similar in all cell lines but IPC 164, which had a lower quantity of this molecule (Fig. 2). Since the relative level of β2m took into account the quantitative expression of Ia-like antigens between the cell lines under the conditions of the radioimmunoassay, it appears that the amount of Ia-like antigens was relatively uniform in all cell lines except IPC 164, on which the amount of those antigens was clearly lower.

Allotypic expression of Ia-like antigens detected using cross-reactive murine anti-Ia mAbs

The results obtained with radioimmunoassay using ten cross-reactive anti-Ia mAbs indicated that the relative percentage of bound antibodies was identical in both pairs of autologous cell lines (Fig. 3).

DISCUSSION

Prevailing data indicate heterogeneous expression of Ia-like antigens in human melanomas [2–7], and a relationship with certain biological properties of malignant melanocytes has been suggested [2, 10, 15]. In this report, Ia-like antigen expression was compared in seven established human melanoma cell lines which differed by

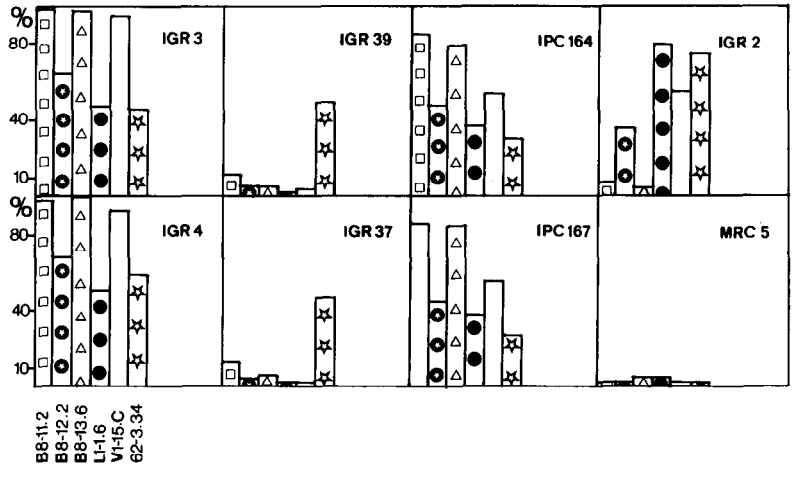

Fig. 1. ^{125}I-labeled (Fab')$_2$ radioimmunoassay of anti-human Ia-like mAbs. Subconfluent cell monolayers were incubated with excess anti-human Ia mAb and then ^{125}I-labeled goat anti-mouse (Fab')$_2$. Results are given as percentages relative to the level of anti-β2m mAb binding, which was taken as 100 under the assay conditions. Values represent the average of at least 3 separate experiments, each in triplicate samples with usually SE ≤10%. For each cell line 6 bars are shown, representing, from left to right, mAbs B8-11.2, B8.12.2, B8.13.6, L1-1.6, V1-15.C and 62.3.34.

origin, degree of tumorigenicity and level of pigmentation, using a large collection of anti-human Ia-like and cross-reactive murine anti-Ia mAbs. Although initially described as specific for HLA-DR antigens, recent data [19, 21–23] suggest that some mAbs used here recognized epitopes present in other molecules, such as the MT/MB/DC, SB and DS antigens [25–28] encoded by the D region of the human major histocompatibility complex. The precise characterization of molecules detected by the various mAbs used in this study has not yet been performed.

The properties of each cell line, particularly the binding of different mAbs, were specific and constant during this study. Stable expression of Ia-like antigens in human melanoma cell lines was also noticed by Winchester *et al.* [5]. All seven cell lines studied reacted with anti-human Ia-like and murine anti-Ia mAbs. We found Ia-like antigens in cell lines initiated from nodular melanomas with different degree of invasion, superficial spreading melanomas or metastatic lymph nodes. Similarly, Ia-like antigens were recently detected in all surgically removed melanomas, which included primary, recurrent and metastatic tumors [7]. DR antigens were also observed on frozen tissue sections of all lesions in the melanocytic neoplastic system [11]. In contrast, cell lines derived from various cutaneous melanoma metastatic nodules of the same patient showed phenotypic difference in the expression of DR antigens [29].

Comparison between the cell lines clearly showed that the reactivity of various mAbs was qualitatively identical in autologous cell lines. This expression of Ia-like antigens with the same allospecificity in cell lines of the same individual origin was in concordance with the presence of identical DR alloantigens previously observed between melanoma cell lines and autologous peripheral B lymphocytes [3].

Conversely, the quantitative expression of β2m and Ia-like antigens was similar in six cell lines but the amounts of these molecules were lower in one cell line. Our results are consistent with the concomitant absence of DR antigens and β2m previously observed in one melanoma cell line [3]. Recently, levels of expression of β2m [5, 30], HLA-A,B and DR antigens [31] were also found to be relatively uniform in human melanoma cell lines.

Large differences in the ability of our cell lines to grow in nude mice were observed. However, Ia-like antigen expression was irrespective of whether cell lines exhibited a weak, intermediate or high degree of tumorigenicity. Moreover, the amount of Ia-like antigens in our cell lines was unrelated to the pigmentation level. Similarly, DR antigens were reported recently as independent of [7, 29] or not directly related to [2] pigmentation of human melanoma

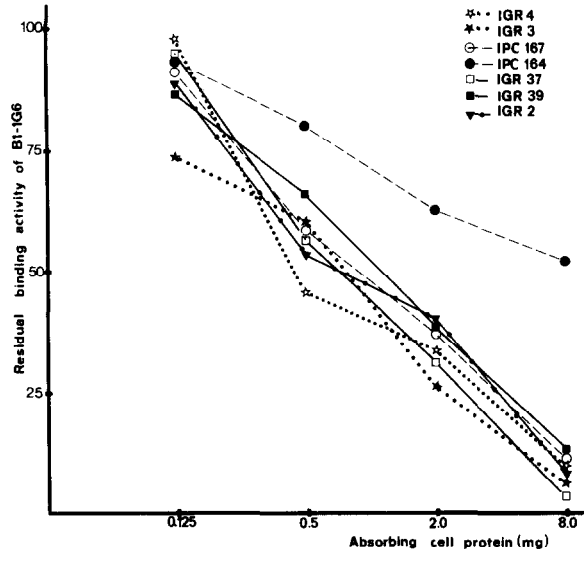

Fig. 2. Quantitative expression of β2m on human melanoma cell lines. Aliquots (0.4 μg in 100 μl) of purified mAb B1-1G6 were absorbed with increasing numbers of whole cells from the different cell lines studied, corresponding to 0.125, 0.5, 2.0 and 8.0 mg of cell proteins. Then aliquots were tested for residual binding activity using radioimmunoassay against the IGR 37 cell line as target cells. The results were expressed as percentage of fixation compared with the binding obtained with unabsorbed mAb, the SE being ±10%.

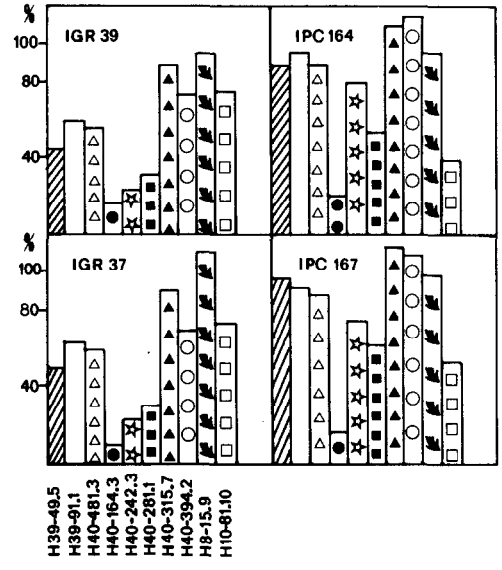

Fig. 3 Expression of Ia-like antigens by indirect radioimmunoassay with cross-reactive murine anti-Ia mAbs. The binding of ten murine anti-Ia mAbs, cross-reactive with Ia-like antigens, was analyzed on two pairs of cell lines respectively initiated from the same patients using cell monolayer radioimmunoassay in the conditions indicated in Fig. 1.

The specificity and the level of fixation of the mAbs in the cell lines determined by radioimmunoassay were corroborated by indirect immunofluorescence assay (data not shown). In addition, this technique enabled detection of Ia-like antigens with a variable intensity on all the cells within each cell line. Such heterogeneity of staining has been reported [7, 11] and may be ascribed to cell cycle fluctuations of Ia-like antigen expression [31–33].

In conclusion, the expression of Ia-like antigens was independent of the primary or metastatic origin, level of pigmentation and degree of tumorigenicity of the human melanoma cell lines. From a practical standpoint, detection of Ia-like antigens can provide an important adjunct to other methods to distinguish malignant melanomas in case of controversial histopathological diagnosis, since these molecules are absent in benign melanocytes [1, 7, 34].

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