HETEROGENEITY IN SURFACE ANTIGEN AND GLYCOPROTEIN EXPRESSION OF CELL LINES DERIVED FROM DIFFERENT MELANOMA METASTASES OF THE SAME PATIENT

Implications for the Study of Tumor Antigens*

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Over the past several years, we have been analyzing the reactivity of human sera for cell surface antigens of malignant melanoma and other human tumors (1–10). The major focus of this work has been to determine whether patients develop antibodies that have specificity for autologous tumor cells. The approach that we developed to answer this question has been referred to as autologous typing and depends on (*a*) cultured lines of tumor cells as target cells, (*b*) the use of several serological techniques to identify different classes of immunoglobulin, and (*c*) absorption tests to determine the specificity of the reactions observed. In this way, autologous antibodies have defined three classes of tumor antigens. Class 1 antigens are of the greatest interest, as they show an absolute restriction to autologous tumors and are found on no other normal or malignant cell type. Class 2 antigens are shared tumor antigens and are found on autologous as well as certain allogeneic tumor cells; recent work indicates that some class 2 antigens may also be found on a restricted range of normal cells and therefore should be classified as autoantigenic differentiation antigens.[1] By far, the greatest number of reactions found in autologous typing are due to antibodies to class 3 antigens. These antigens are found on a wide variety of normal and malignant cells of human and animal origin. Some class 3 reactivity can be traced to heterologous serum components used in the culturing of cells, the antigens disappearing when the cells are grown in human serum. Other class 3 antigens appear to be autoantigenic components that are widely distributed on adult and fetal cells. Absorption tests have been critical as a means to distinguish the class of antigens detected by autologous typing.

To date, three class 1 antigens have been defined on human melanoma cells, the tumor type that has been most extensively investigated: AU antigen (1, 7), BD antigen (2), and BI antigen (3, 11). In this report, we describe a new class 1 melanoma antigen,

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DX. This analysis has been particularly instructive with regard to the detection of class 1 antigens and may explain why class 1 reactivity is apparently so uncommon in melanoma patients. Several melanoma cell lines were derived from separate metastatic foci of the same patient (DX) and each could be distinguished from one another on the basis of stable phenotypic difference in growth, morphology, and in the expression of cell surface antigens and glycoproteins. Only one of the lines expressed sufficient levels of DX antigen to allow detection of autologous DX antibody.

Materials and Methods

Tissue Culture. Melanoma and other cell lines were derived as described previously (1, 5, 6). Two human fetal cell lines (Flow 4000 and Flow 5000) were purchased from Flow Laboratories (Rockville, Md.). B cell lines from patients with melanoma were derived from peripheral blood lymphocytes transformed by Epstein-Barr virus released from the B-95-8 marmoset lymphoid line.

Cultures were maintained in Eagle's minimum essential medium supplemented with 2 mM glutamine, 1% nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (FBS).[2] To remove heterologous FBS components, melanoma cells were cultured in prescreened 10% human sera from AB blood type donors for at least 6 wk (generally three subcultures). Cultures were regularly tested for mycoplasma, fungi, and bacteria, and contaminated cultures were discarded.

Serological Procedures. The protein A hemadsorption (PA) and immune adherence assays (IA) were performed as described previously (2, 5). For anti-human γ-heavy chain assays (anti-γG) indicator cells were prepared by conjugating the immunoglobulin fraction of rabbit anti-human γ-heavy chain (DAKO, Copenhagen) to human O^+ erythrocytes with 0.01% chromium chloride (8). Assays were performed in Falcon 3040 microtest II plates (Falcon Labware, Oxnard, Calif.) Target cells (plated 1–2 d previously) and human sera were incubated for 1 h at 37°C (pA and anti-γG) or 4°C (IA). After washing the target cells, indicator cells were added and incubated with target cells for 1 h (PA and anti-γG assay) or 30 min (IA). The plates were then washed gently and reactions evaluated under light microscopy. When testing cells grown in medium containing human serum, 2% (wt/vol) gamma globulin-free human serum albumin (Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline solution was substituted for the 5% gamma globulin-free bovine serum regularly used in the serological assays. Qualitative absorption tests were performed by absorbing 30–50 μl of serum (diluted according to the end point) with an equal volume of washed packed cells for 1 h at room temperature and testing for residual antibody on target cells grown in Falcon 3034 plates (Falcon Labware) (6).

Specific Labeling and Analysis of Cell Surface Glycoproteins. Cells were labeled by three procedures, as described previously (12): (*a*) neuraminidase-galactose oxidase-[^{3}H]borohydride, (*b*) galactose oxidase-[^{3}H]borohydride, and (*c*) dilute periodate-[^{3}H]borohydride. Labeled glycoproteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography (13).

Results

Clinical History of Patient DX. DX is a 70-yr-old white female who presented with an enlarging pigmented nevus on the distal third of the left leg in June 1975. She was treated surgically by wide excision and prophylactic inguinal lymph node dissection. Pathology showed a Clark's level III malignant melanoma with four satellite lesions and no evidence of disease in regional lymph nodes. A metastatic lesion in the subcutaneous tissue of the left thigh was excised in September 1978 and a melanoma cell line (designated DX-1) was established from the surgical specimen. 1 mo later,

[2] *Abbreviations used in this paper:* anti-γG, anti-human γ-heavy chain assay; FBS, fetal bovine serum; IA, immune adherence assay; PA, protein A hemadsorption assay; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

four additional subcutaneous metastases appeared on the thigh. The patient received triweekly subcutaneous vaccinations with irradiated allogeneic melanoma cells, SK-MEL-13, continuing from November 1978 to July 1979 with no change in the size of metastases. All visible melanoma lesions were excised in August 1979 and two new melanoma cell lines from two discrete metastatic deposits were established in tissue culture (DX-2 and DX-3). Vaccination with irradiated DX-1 melanoma cells was carried out from November to December 1979. She remained in good health, free of evident tumor, until August 1980 when she developed new lesions in the left inguinal area. At present, she has multiple soft tissue metastases.

Morphology and Growth Characteristics of Melanoma Cell Lines Derived from Patient DX. Fig. 1 illustrates the characteristic morphology of the three melanoma cell lines (DX-1, DX-2, DX-3) established from three distinct subcutaneous metastases. DX-1 and DX-2 cells accumulate visible pigmentation; DX-3 cells appear unpigmented. The DX-2 and DX-3 cell lines grow as attached monolayers, whereas the DX-1 line has a marked tendency to grow in suspension as microcolonies of rounded cells after initial attachment. This growth trait of the DX-1 line has been stable over 25 passage generations, and both suspension cells and monolayer DX-1 cells can initiate cultures in which the cells first attach to the substrate and then progressively detach over the next 7–14 d. DX-2 cells assume a characteristic spindle shape during growth and

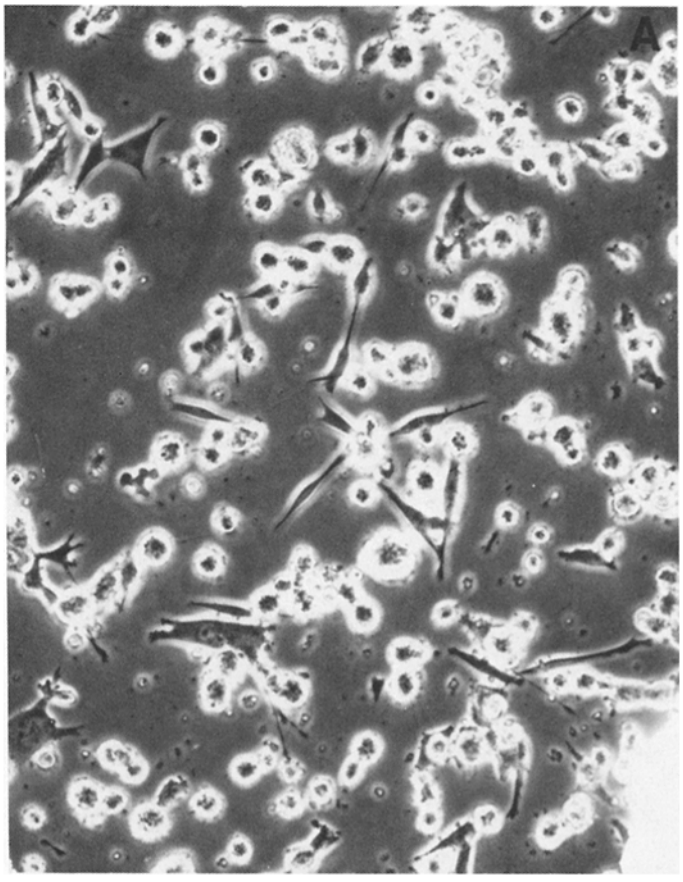

FIG. 1 A. Photomicrograph of cell line DX-1 (×220). See text for description.

FIG. 1 B. Photomicrograph of cell line DX-2 (×220). See text for description.

form multiple layers of cells. DX-3 cells show an epithelial type of morphology and tend to be more sensitive to contact inhibition than DX-2 cells. The doubling time and saturation density of DX-1, DX-2, and DX-3 cell lines were 70 h and 35,000 cells/cm^2 (monolayer plus suspension cells), 50 h and 44,000 cells/cm^2, and 61 h and 40,000 cells/cm^2, respectively.

The HLA haplotype of DX-1, DX-2, and DX-3 was identical and corresponded to the patient's HLA haplotype as determined by lymphocyte typing (14): A-2, w-31, Bw-21, w-35, and Cw-4. In addition, isoenzyme markers for five traits (glyoxalase I, phosphoglucomutase-1, phosphoglucomutase-3, esterase D, and glutamate-oxaloacetate transaminase) revealed no discrepancy between the phenotypes of the three DX melanoma cell lines.

Typing of DX-1, DX-2, and DX-3 Cells for HLA-DR and Other Cell Surface Antigens. Table I summarizes the results of serological tests using a battery of conventional and monoclonal reagents identifying differentiation antigens on the surface of melanoma cells (8, 15–17). Expression of HLA-DR determinants showed the most striking difference. DX-1 and DX-3 express HLA-DR products, whereas DX-2 does not. The lack of detectable HLA-DR determinants on the DX-2 line was confirmed by absorption analysis (Fig. 2) and by radioimmunoprecipitation experi-

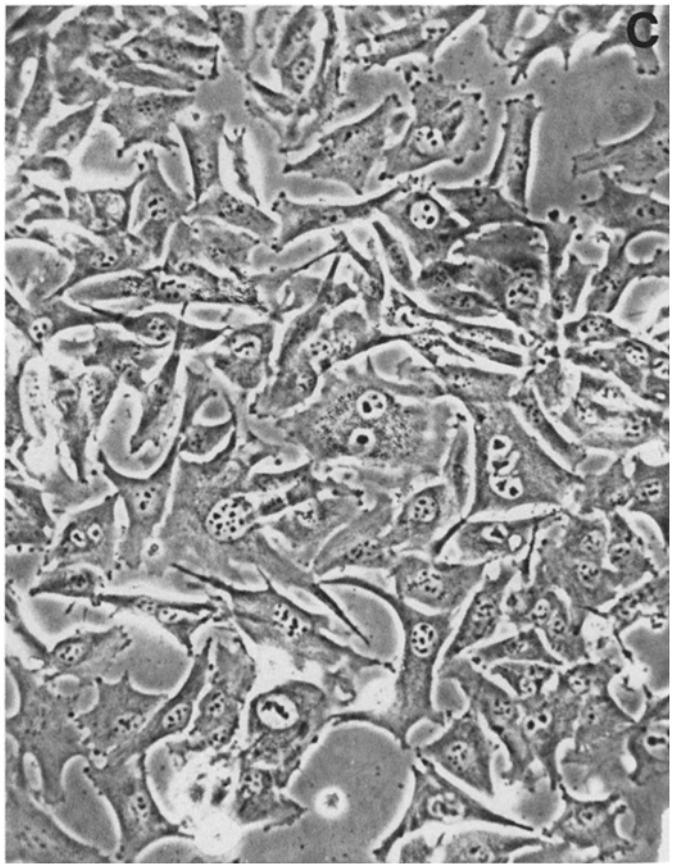

FIG. 1 C. Photomicrograph of cell line DX-3 (×220). See text for description.

ments (see below). Less marked, but characteristic differences were detected in the expression of a number of other cell surface antigens (Table I).

Glycoprotein Profiles of DX Melanoma Lines Detected by Cell Surface Labeling Procedures. Melanomas express a characteristic pattern of cell surface glycoproteins that can be detected by surface radiolabeling procedures (12). All melanoma cell lines have two major groups of glycoproteins with molecular weights of ~90,000 and 120,000. These glycoproteins are so highly sialylated that they cannot be labeled by galactose-oxidase-[^{3}H]borohydride without prior neuraminidase treatment; this property is a peculiarity of melanoma cells which distinguishes them from other cell types. Application of this approach to DX melanoma has demonstrated differences in the surface characteristic of the three DX cell lines (Fig. 3). DX-2 and DX-3 have rather similar profiles of cell surface glycoproteins in the high molecular weight region, with the components of 90,000 and 120,000 mol wt being particularly prominent. As expected, these components were not labeled by galactose-oxidase-[^{3}H]borohydride without prior neuraminidase treatment. DX-2 had an additional prominent component of 60,000 mol wt which was only weakly labeled in DX-3. DX-3 differed from DX-2 in having two major glycoproteins in the low molecular weight region of 32,000

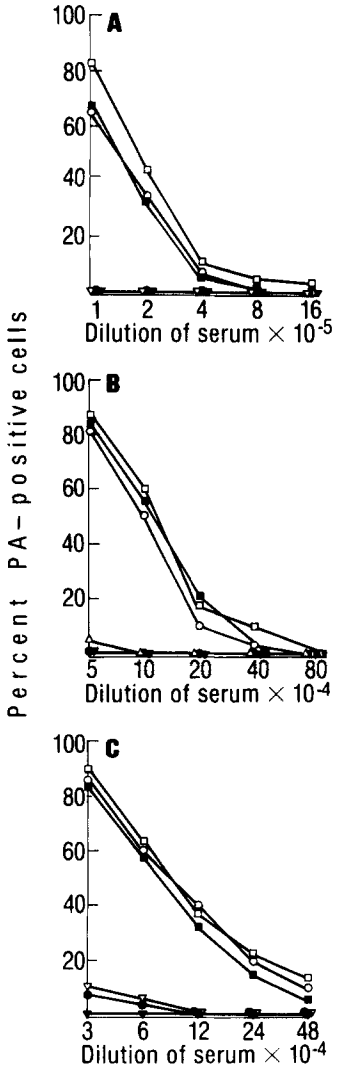

FIG. 2. HLA-DR phenotype of DX-1, DX-2, and DX-3 melanoma cell lines as determined by absorption analysis. A, rabbit anti-HLA-DR serum; B, human-anti-HLA-DR serum; C, mouse monoclonal anti-HLA-DR serum. Typing sera (see Table I) diluted according to end point were absorbed with equal volumes of the indicted cell types. Residual reactivity of recovered serum was tested on SK-MEL-93 (an HLA-DR-expressing melanoma cell line). DX-1, DX-3, and SK-MEL-28 absorbed HLA-DR reactivity. DX-2 and SK-MEL-28 Clone A (a non-HLA-DR-expressing cloned variant) did not absorb HLA-DR reactivity. ○, DX-2; ●, DX-1; ▼, DX-3; ▽, SK-MEL-28; ■, SK-MEL-28 Clone A; □, unabsorbed serum.

and 27,000. Using specific antisera, these components were shown to be HLA-DR subunits, thus confirming the serological data presented above. DX-1 monolayer cells demonstrated unusual surface-labeling characteristics in comparison with DX-1 suspension cells, DX-2, and DX-3 cells. Using the neuraminidase-galactose-oxidase-[^{3}H] borohydride procedure, only one major glycoprotein of 90,000 mol wt was labeled in DX-1 monolayer cells; treatment with galactose-oxidase-[^{3}H]borohydride without neuraminidase did not label any specific components. The DX-1 monolayer cells

TABLE I

Serological Typing for Cell Surface Antigens Expressed by Three Melanoma Cell Lines Derived from Patient DX

Antigen system detected	Source of antibody*	Reference or description	Cell lines		
			DX-1	DX-2	DX-3
			Serum titers × 10^{-3}		
HLA-DR	Rabbit antiserum	18	64	0	256
HLA-DR	Human serum	Transfused individual	50	0	300
HLA-DR	Mouse monoclonal	18, 19	50	0	500
HLA-A, B, C	Mouse monoclonal	20	320	1,200	320
Mel-1	Human serum	8	3	3	0.4
Q_{24} (gp150)	Mouse monoclonal	15	1,000	4,000	1,000
M_{17} (gp95)	Mouse monoclonal	15	50	3,000	250
O_5	Mouse monoclonal	15	1,500	1,500	625
R_{24}	Mouse monoclonal	15	3	100	3
R_8	Mouse monoclonal	15	1,000	3,000	3,000
D_{14}	Mouse monoclonal	Mouse immunized with	50	500	25
E_{20}	Mouse monoclonal	DX-2 melanoma cell line	256	1,000	1,000
A_{27}	Mouse monoclonal	Mouse immunized with SK-	8	12	12
A_{123}	Mouse monoclonal	MEL-19 melanoma cell line	1	25	50
A_{124}	Mouse monoclonal		8	200	12
L_{166}	Mouse monoclonal	Mouse immunized with SK-MEL-33 melanoma cell line	50	800	50
MCT_{138}	Mouse monoclonal	Mouse immunized with cultured human cutaneous melanocytes	5,000	10,000	50

* Mouse monoclonal reagents: sera or ascites fluid of *nu/nu* mice.

labeled very poorly with periodate-[^{3}H]borohydride, also in contrast to DX-1 suspension cells, DX-2, and DX-3 cells. Since periodate, in the concentration used, selectively oxidizes sialic acid, the latter results would suggest that the surface glycoproteins of DX-1 monolayer cells have very few sialic acid residues. On the other hand, hydrolysis with neuraminidase clearly removes sialic acid to expose galactose residues that are subsequently labeled with [^{3}H]borohydride. A possible explanation is that DX-1 monolayer cells have substituted sialic acid residues that are not oxidized by periodate but can be hydrolyzed by neuraminidase. This possibility needs further investigation, but whatever the explanation, the results demonstrate that DX-1 monolayer cells have surface characteristics quite different from those of DX-1 suspension cells.

Autologous Typing with Sera from Patient DX

DIRECT SEROLOGICAL TESTS. Sera from patient DX were tested for antibody reacting with surface antigens of autologous DX-1, DX-2, and DX-3 cells. Autologous reactions were seen with DX-2 target cells, but not with DX-1 or DX-3 cells, or with DX fibroblasts. Fig. 4 shows the reactions of DX serum obtained in September 1978

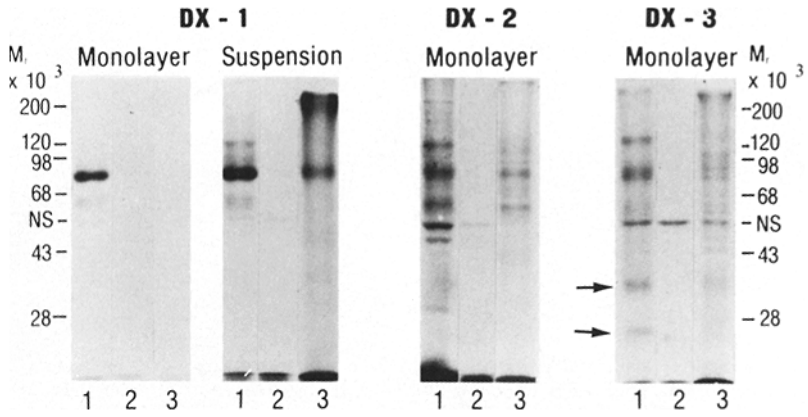

FIG. 3. Fluorograms of SDS-PAGE of DX melanoma cell lines labeled by treatment with 1, neuraminidase-galactose-oxidase-[^{3}H]borohydride, 2, galactose oxidase-[^{3}H]borohydride, and 3, periodate-[^{3}H]borohydride. Molecular weight standards: myosin, 200,000; β-galactosidase, 120,000; phosphorylase, 98,000; bovine serum albumin, 68,000; ovalbumin, 43,000; concanavalin A, 28,000. NS, nonspecific component labeled by [^{3}H]borohydride alone. Arrows indicate HLA-DR subunits.

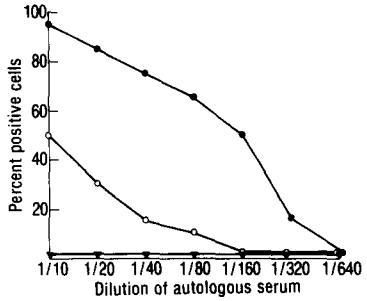

FIG. 4. Reactivity of DX serum for autologous DX-2 melanoma cells as determined by three serological assays. ●, PA; ▼, IA; ○, anti-γG.

with DX-2 cells as determined in three serological test systems. Antibody was detected in PA and anti-γG, but not in IA. Ion-exchange chromatography of DX sera showed that all reactivity for DX-2 cells resided in the IgG fraction. As PA gave highest titers with DX sera, all further analysis was done with this assay.

TIME-COURSE OF DX AUTOLOGOUS REACTIVITY. Fig. 5 shows the PA reactivity of a sequential series of specimens of DX sera for DX-2 melanoma cells. No reactions were seen with the initial serum specimens. Antibody was first detected in sera obtained in September 1978 and peak levels were found in January 1979. Reactivity then began to fall, and for the past 17 mo no autologous reactivity has been detected. Vaccination with allogeneic or autologous melanoma cells (see above) appeared to have no influence on antibody levels.

ABSORPTION ANALYSIS OF DX SERA; DEFINITION OF DX ANTIGEN. The reactivity of DX sera (obtained September 1978) for DX-2 cells was analyzed by qualitative absorption tests. Fig. 6 illustrates an absorption test and Table II summarizes the results of the analysis. Only DX-2 cells absorbed autologous reactivity completely. In some tests, DX-1 and DX-3 cells gave a pattern of partial absorption, suggesting that the cells express the same antigenic determinants detected by DX serum on DX-2

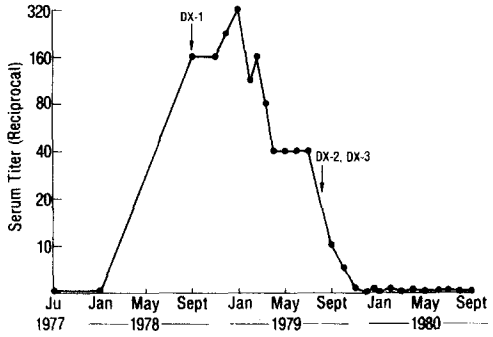

FIG. 5. Reactivity of sequential serum specimens from patient DX for DX-2 melanoma cells. Serological assay: PA. Titer refers to serum dilution showing 50% positive target cells. Arrows indicate dates that DX-1, DX-2, and DX-3 melanoma cell lines were initiated from biopsy specimens.

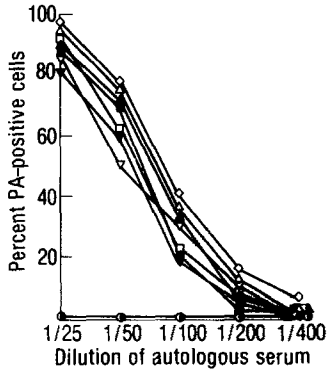

FIG. 6. Qualitative absorption analysis of the PA reactivity of DX serum for DX-2 melanoma cells. DX reactivity was completely absorbed by DX-2 melanoma cells grown in FBS or in normal human serum. DX-1, DX-3, SK-MEL-13, SK-MEL-28, DX normal skin fibroblasts and DX EBV-transformed B cells did not absorb DX reactivity. ○, DX-2 (FCS); ●, DX-2 (HS); ▼, DX-1; ▽, DX-3; ■, DX normal skin fibroblasts; □, DX EBV-transformed B cells; △, SK-MEL-13; ▲, SK-MEL-28; ◇, unabsorbed serum.

cells, but at a quantitatively lower level. To investigate this possibility, double absorptions of DX sera were carried out. Fig. 7 shows that two successive serum absorptions with DX-1 or DX-3 removed reactivity for DX-2 cells; double absorptions with eight allogeneic melanoma cell lines did not reduce DX reactivity.

As shown in Table II, no cell type other than DX-1, DX-2, or DX-3 absorbed autologous reactivity. These tests included 45 cell lines of allogeneic melanoma, and a wide range of cell types other than melanoma. Absorption with DX autologous skin fibroblasts and Epstein-Barr virus (EBV)-transformed B cells did not remove autologous reactivity. To exclude the participation of antigens related to FBS in the observed reactions, DX-2 melanoma cells were grown for at least four passages in medium supplemented with normal human serum (HS) only. As seen in Fig. 6, these cells absorbed autologous reactivity as efficiently as cells grown in FBS. The results indicate the detection of a class 1 melanoma antigen, which we have designated DX, restricted to autologous DX melanoma cells.

ALLOGENEIC TYPING WITH DX SERA. DX sera reacted in direct tests with 9–10

TABLE II

Summary of Results of Absorption Analysis of the PA Reactivity of DX Serum for Autologous Melanoma Cells

Positive absorption	Negative absorption	
Autologous cultured melanoma cells:	Autologous cultured cells:	Allogeneic cultured nonmelanoma cells:
DX-2 (FBS)	Skin fibroblasts	Astrocytoma: U-373, U-138, AN, AS
DX-2 (Human serum)	EBV-transformed B cells	Breast Cancer: MCF-7, BT-20, AlAb
	Allogeneic cultured melanoma cells:	Renal Cancer: SK-RC-1, SK-RC-2, SK-RC-6, SK-RC-8, SK-RC-9, SK-RC-10, Caki-1
Partial Absorption	SK-MEL-13 SK-MEL-72 SK-MEL-132	Other cancers: HT-29 (colon), ME-180 (cervix), SK-GR-1 (testicular), SK-LC-LL (lung), 5627 (bladder), MOLT-4 (leukemia)
	SK-MEL-18 SK-MEL-73 SK-MEL-133	
	SK-MEL-19 SK-MEL-75 SK-MEL-140	
	SK-MEL-20 SK-MEL-78 SK-MEL-147	
DX-1	SK-MEL-23 SK-MEL-79 MeWo	EBV-transformed B cells: BF, CS, BD, AV, DS, AE
DX-3	SK-MEL-27 SK-MEL-80	
	SK-MEL-28 SK-MEL-94	Skin fibroblasts: AS, BE, EM, ER, FB
	SK-MEL-29 SK-MEL-95	Fetal cells: skin, choroid, brain, liver, Flow 4000, Flow 5000
	SK-MEL-30 SK-MEL-100	
	SK-MEL-31 SK-MEL-101	Allogeneic noncultured cells:
	SK-MEL-33 SK-MEL-110	A, B, O erythrocytes, adult brain
	SK-MEL-37 SK-MEL-118	Xenogeneic cells and serum:
	SK-MEL-40 SK-MEL-119	VERO (monkey kidney cell line), BHK/21 (hamster kidney cell line), BALB/3T3 (mouse cell line), sheep erythrocytes, fetal bovine serum
	SK-MEL-42 SK-MEL-122	
	SK-MEL-56 SK-MEL-123	
	SK-MEL-57 SK-MEL-125	
	SK-MEL-60 SK-MEL-127	
	SK-MEL-61 SK-MEL-129	
	SK-MEL-63 SK-MEL-130	
	SK-MEL-64 SK-MEL-131	

allogeneic melanoma cell lines. It seemed most likely that these allogeneic reactions were due to antibodies to HLA-related antigens in DX sera (DX is the mother of four children). If this were the case, EBV-transformed B cells from donors of the allogeneic melanomas should remove all reactivity from DX sera for the allogeneic melanoma cells. In four such pairs of allogeneic B cells and melanoma cells, B cells absorbed all allogeneic DX reactivity, but did not alter autologous reactivity of DX sera for DX-2 cells.

CHARACTERISTICS OF THE DX ANTIGEN. Absorption tests indicate that the DX

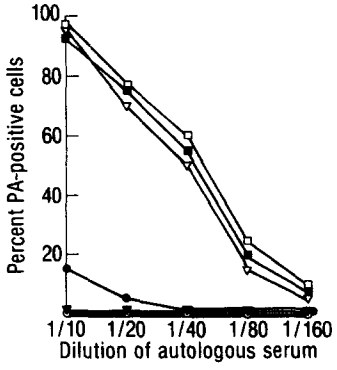

FIG. 7. Effect of double absorptions of DX serum with DX-1, DX-2, or DX-3 melanoma cell lines. Aliquots of 1:20 diluted DX sera were absorbed with equal volumes of the indicated cell types for 30 min at 20°C, then for 30 min at 0°C. After removal of absorbing cells by centrifugation, the recovered DX sera were similarly absorbed a second time with equal volumes of the same cell type. The doubly absorbed DX sera were then tested for residual reactivity for DX-2 melanoma cells. ○, DX-2; ●, DX-1; ▼, DX-3; ▽, SK-MEL-28; ■, SK-MEL-29; □, unabsorbed serum.

determinant is heat labile (100°C for 5 min) and trypsin sensitive. Efforts to immunoprecipitate the antigen from radiolabeled DX-2 cell extracts have been unsuccessful (see Discussion).

SEROLOGICAL COMPARISON OF TWO CLASS 1 ANTIGENS: AU AND DX. AU is the prototype class 1 melanoma antigen (1). To determine the possible serological relatedness of DX and AU antigens, reciprocal absorption tests with DX and AU typing sera were carried out (Fig. 8). DX-2 melanoma removed DX reactivity, but not AU reactivity. AU melanoma (SK-MEL-28) absorbed AU reactivity, but not DX reactivity. Both AU and DX-2 melanoma cells absorbed reactivity from a typing sera detecting the Mel-1 antigen, an antigen related to melanocyte differentiation (8). Another melanoma line (SK-MEL-19) did not absorb DX, AU, or Mel-1 reactivity.

Characteristics of Three Additional Cell Lines from Patient DX. Three additional cell lines have been established from separate metastases removed from patient DX in August

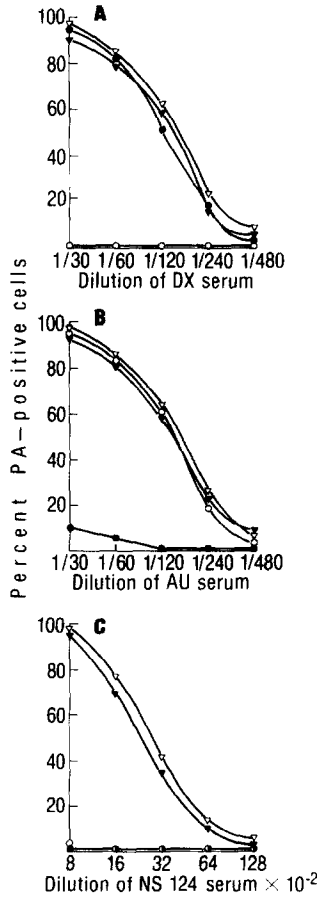

FIG. 8. Serological typing for DX, AU, and Mel-1 antigens. Qualitative absorption was carried out with end point-diluted typing serum absorbed with equal volumes of the indicated cell types. Serological assay: PA. Typing systems: A, DX:DX serum reacted with DX-2 melanoma line; B, AU: AU serum reacted with AU melanoma line (SK-MEL-28); and C, Mel-1: human serum NS 124 reacted with SK-MEL-28 melanoma line. ○, DX-2; ●, SK-MEL-28; ▼, SK-MEL-29; ▽, unabsorbed serum.

1980 (DX-4) and December 1980 (DX-5, DX-6). Table III summarizes the features of these new cell lines in relation to the DX-1, DX-2, and DX-3 cell lines.

Discussion

As a consequence of our interest in the humoral immune response of patients to autologous melanoma cells, we have established melanoma cell lines from over 150 individuals. Virtually all the lines were derived from metastatic disease, and the success rate for establishing melanoma cell lines over the past 6 yr has ranged from 15–40%. (Our experience with primary melanoma has been more limited, primarily because the specimen is usually required in its entirety for microstaging.) A comparison of established melanoma cell lines from different individuals shows striking variation in morphology, growth rate, pigmentation, and pattern of surface antigens and glycoproteins. This phenotypic heterogeneity of cultured melanoma may reflect a corresponding diversity in the phenotype of normal cells undergoing melanocyte differentiation, with malignant transformation fixing a cell within a particular stage in the melanoblast → melanocyte lineage. Additional factors, either genetic or epigenetic, must also be involved in the generation of this diversity, because clones derived from the same melanoma cell line show stable phenotypic differences (21; A. Albino, unpublished observations). This could mean that melanomas are initially derived from more than one clone of transformed cells, or that the disease is in fact initiated by a single transformed clone with stable variants of the clone being generated throughout the course of the disease. Either explanation could account for the phenotypic diversity of cell lines derived from the different metastatic deposits of melanomas removed from patient DX over the course of 2 yr. Although some degree of variation can be expected to result from prolonged in vitro passage, it appears most likely that the differences that distinguish these three lines represent characteristics of major or minor cell populations present in vivo that have emerged as the predominant phenotype in vitro. For instance, DX-2 and DX-3 cell lines, derived from two independent metastatic lesions located some 10 cm apart and removed at the same time, differed morphologically and in the quantitative expression of HLA-DR antigens when tested 20 d after explantation. Similarly, three new cell lines derived from metastatic lesions of this patient (DX-4, DX-5, and DX-6), tested within 2 d of

TABLE III

Characteristics of Six Established Melanoma Cell Lines Derived from Separate Metastases of Patient DX

Cell line	Morphology	Pigmentation	HLA-DR*	Mel-1*	Autologous typing for DX antigen		
					Direct test (titer)	Single absorption‡	Double absorption‡
DX-1	Spindle (monolayer) plus suspension	+	+	+	− (<1:10)	− or ±	+
DX-2	Spindle	+	−	+	+ (1:80–1:320)	+	+
DX-3	Epithelioid	−	+	+	− (<1:10)	− or ±	+
DX-4	Epithelioid	+	−	−	− (<1:10)	−	−
DX-5	Cuboidal	−	+	+	−(<1:10)	−	±
DX-6	Spindle/epithelioid	+	+	+	− (<1:10)	−	±

* As determined in direct serological tests and by absorption analysis.

‡ See Figs. 6 and 7. +, complete absorption; ±, partial absorption; −, no absorption.

explantation, also showed stable phenotypic differences in the expression of HLA-DR antigens.

Analysis of these melanoma cell lines derived from patient DX also clearly illustrates the importance of phenotypic variation in studies of tumor immunity. If DX-1 or DX-3, or the more recently derived DX-4, DX-5, or DX-6 cell lines had been the only targets available to study the autologous reactions of sera from patient DX, the presence of antibody defining a class 1 melanoma antigen would have been missed, since only DX-2 expressed this antigen in sufficient quantity to be detected by sensitive serological assays. In our past studies of humoral immunity to melanoma, we have generally depended on a single target cell line to test the reactivity of autologous sera. Antibodies to class 1 antigens have been found only rarely—in 4 patients, including patient DX, out of a total of 75 patients studied (10). This low frequency of class 1 reactivity may have several explanations, ranging from lack of class 1 antigens on the majority of melanomas, to lack of antibody to melanoma class 1 antigens in the majority of melanoma patients. It is also possible that the serological assays used are of insufficient sensitivity to detect antibodies of low titer, low avidity, or of an unusual subclass, or that cells expressing class 1 antigens have a selective disadvantage in tissue culture. Experience with the six melanoma lines derived from patient DX emphasizes the critical nature of the target cell in identifying class 1 antigens and suggests that class 1 reactivity may in fact be more common than our past studies with only a single cell line from each melanoma patient would indicate. We are investigating this possibility by establishing several cell lines from each new melanoma patient, either from individual metastases, as in the case of DX, or from different clones isolated directly from the same melanoma specimen.

With the serological identification of four melanoma antigens of the class 1 type, certain similarities have become evident. By definition, each shows an absolute restriction to autologous melanoma cells, not being found on autologous normal cells or any normal or malignant cell of allogeneic or xenogeneic origin. Antibodies detecting three class 1 antigens (AU, BI, and DX) belong to the IgG class, whereas antibody to the BD antigen is IgM. AU, the prototype class 1 antigen, has been characterized as a glycoprotein, as indicated by its affinity for *Lens culinaris* lectin (7). It is solubilized by papain, and molecular sizing of the solubilized AU antigen shows it to have a molecular weight in the range of 25,000–40,000. From the studies done to date, DX and BI antigens also have characteristics of glycoproteins. Despite relatively high titers of sera detecting AU, BI, and DX antigens, it has not been possible to immunoprecipitate radiolabeled cell extracts with these human antibodies as a way to characterize class 1 antigens further. For this reason, much effort is now going into preparing mouse or human monoclonal antibody to these antigens. The key question is whether class 1 antigens are closely related products coded for by the same locus or structurally unrelated products coded for by distinct loci. Purification and biochemical characterization of a series of class 1 antigens and mapping of their coding genes by somatic cell genetics and gene cloning should provide ways to address this question.

Summary

Three established lines of melanoma cells were derived from anatomically distinct metastases occurring in a single patient (DX). The lines, DX-1, DX-2, and DX-3, showed marked phenotypic diversity, as indicated by characteristic differences in

growth rate, morphology, pigmentation, and the expression of surface antigens and glycoproteins. DX-1 and DX-3 expressed HLA-DR products, whereas DX-2 lacked HLA-DR expression. DX-1, DX-2, and DX-3 could also be distinguished on the basis of the profile of radiolabeled glycoproteins. Additional quantitative differences in the surface antigenic phenotype of the three cell lines were revealed by serological tests with a battery of monoclonal and conventional antibodies defining melanoma differentiation antigens. In tests for autologous humoral immunity to melanoma cells, sera from patient DX were found to have IgG antibody that reacted with surface antigens of DX-2 cells; no autologous reactivity was seen with DX-1 or DX-3 target cells or with three more recently established melanoma cell lines from patient DX. Absorption analysis indicated that the antigen detected by DX sera on DX-2 cells is a class 1 melanoma antigen, having been detected only on DX-2 cells and in much lower but demonstrable amounts on DX-1 and DX-3 cells. No other cell type, including DX normal fibroblasts, DX B cells, or 45 allogeneic melanoma cell lines expressed the class 1 antigen of DX melanoma. The fact that only one of the melanoma cell lines derived from patient DX was a suitable target for the detection of autologous class 1 reactivity has implications for the study of human tumor antigens and may explain why antibody to class 1 antigens has been found so infrequently in past studies of melanoma patients.

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