CLASS II HISTOCOMPATIBILITY ANTIGEN EXPRESSION IN HUMAN MELANOCYTES TRANSFORMED BY HARVEY MURINE SARCOMA VIRUS (Ha-MSV) AND KIRSTEN MSV RETROVIRUSES

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Although human Ia or class II histocompatibility antigens were initially thought to be restricted to cells of the immune system, studies have shown the presence of Ia antigens on malignant melanomas (1–3) and other cell types having no known immune functions (3–6). While Ia antigens can be detected on most melanoma biopsy specimens and melanoma cell lines (7, 8), normal melanocytes lack constitutive Ia expression (9), either in vivo or in vitro. To investigate the relationship of Ia expression in melanomas to malignant transformation, we infected melanocytes with transforming amphotropic pseudotypes of Harvey murine sarcoma virus (Ha-MSV)[1] or Kirsten murine sarcoma virus (Ki-MSV). The Ha-MSV and Ki-MSV retroviruses contain oncogenes of the *ras* gene family, and were chosen because our previous studies (10) indicated that 10% of cultured melanomas have an activated *ras* gene allele (either Ha-*ras* or N-*ras*), and no other conclusive perturbation (i.e., rearrangement or amplification) in 15 other known oncogenes (A. P. Albino, unpublished results).

Materials and Methods

Tissue Culture. Melanocytes and other cell lines were derived as described previously (3). Cultures were maintained in Eagle's MEM supplemented with 2 mM glutamine, 1% nonessential amino acids, 100 U/ml penicillin and 10% FCS. For culturing melanocytes, the medium was supplemented (11) with 10^{-8} M cholera toxin (Schwartz/Mann Biologicals, Orangeburg, NY) and 10 ng/ml 12-*o*-tetradecanoyl phorbol-13-acetate (TPA) (Consolidated Midland Corp., Brewster, NY). Melanocyte cultures were used during early passages (less than four).

Virological Techniques. Viral stocks were isolated from NIH 3T3 cells infected with 4070A amphotropic murine leukemia virus (MuLV) (12), or from Ki-MSV, Ha-MSV, or Ki-MSV temperature sensitive (ts) mutant–infected NIH 3T3 nonproducer clones superinfected with 4070A amphotropic helper MuLV (13, 14, and A. I. Oliff, unpublished data). The Ki-MSV and Ha-MSV pseudotypes were collected as fresh 24-h cell-free supernatant fluids and frozen at −70°C until use. ~10^5 melanocytes were pretreated for 60 min with DEAE/dextran (25 μg/ml), washed, then incubated with virus at a multiplicity

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[1] *Abbreviations used in this paper:* Ha, Harvey; Ki, Kirsten; MSV, murine sarcoma virus; MuLV, murine leukemia virus; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; ts, temperature-sensitive.

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of infection of one. Transforming activity of virus produced by infected cells was determined by focus assays on NIH 3T3 cells (15). Infectious virus produced by melanocytes infected with 4070A amphotropic virus was quantitated by titrating the virus on NIH 3T3 cells and determining the expression of MuLV p30 *gag* gene protein in the cytoplasm. The ability of infected cells to form growing colonies in soft agar (16) and to form infectious centers on NIH 3T3 cells (17) were determined as described. Mouse NIH 3T3 cells infected with the Ki-MSV ts mutant showed a 10^4-fold decrease in focus formation at the nonpermissive temperature.

Northern Blot Analysis. Total RNA was prepared as described (18), fractionated by electrophoresis on a 1.0% agarose gel (3 μg/lane) in the presence of formaldehyde (19), and transferred to Nytran membranes (Schleicher and Shuell, Keene, NH). Probes were ^{32}P-labeled and hybridized to membranes as described (10).

Serological Reagents. The following mouse mAb were used for serological testing: L243 (recognizes a nonpolymorphic determinant on human Ia molecules [20]), W6/32 (recognizes monomorphic determinants on HLA-A,B,C molecules [21]), C350 (recognizes a cell-surface antigenic determinant expressed only by pigmented cells; A. N. Houghton, T. Thomson, and F. Real, unpublished results), CF21 and TA99 (recognize antigenic determinants present in mature melanosomes [22, 23]), R24 (recognizes G_{D3} ganglioside [24]), B5.2 (recognizes a chondroitin sulfated proteoglycan [8]), and B2.6 (recognizes an antigenic determinant present on the cell surface of a subset of melanomas and astrocytomas; A. P. Albino and G. Cairncross, unpublished results). Human serum containing a natural antibody detecting the MEL-1 differentiation antigen of melanomas and melanocytes has been reported (25). Rabbit anti-Rauscher MuLV group-specific p30 serum (26) and rat anti-p21 mAb Y13-259 (27) were generous gifts of Drs. Paul V. O'Donnell and Mark E. Furth, respectively, of Sloan-Kettering Memorial Cancer Center.

Immunoprecipitation Analysis. Cells were radiolabeled by metabolic incorporation of [^{35}S]methionine (New England Nuclear, Boston, MA: 1,000 Ci/mmol) using 250 μCi in 10 ml of methionine-free MEM containing 1% FCS for 16 h. Labeled cells were extracted as described (10). Immunoprecipitation was carried out by mixing a portion of the cell extract (10×10^6 cpm) with 1 μl of undiluted ascites fluid Y13-259 (anti-p21), W6/32 (anti-HLA-A,B,C) or L243 (anti-Ia) and 15 μl of rabbit anti-mouse immunoglobulin serum (Cappel Laboratories, Cochranville, PA). Immune complexes were isolated with protein A–Sepharose C14B (Pharmacia Fine Chemicals, Piscataway, NJ) and the labeled components were detected by SDS-PAGE and fluorography as described (10).

Serological Assays. The protein A and anti-mouse immunoglobulin hemadsorption assays were performed as described (3). Indicator cells were prepared by conjugating either protein A (Pharmacia Fine Chemicals) or the immunoglobulin fraction of rabbit anti-mouse γ heavy chain (Dako Corp., Santa Barbara, CA) to human O^+ erythrocytes with 0.01% chromium chloride. Assays were performed in Falcon 3040 microtest plates (Falcon Labware, Oxnard, CA). Target cells (plated 1–2 d previously) and serial antibody dilutions were incubated for 1 h at 37°C. Target cells were then washed and indicator cells were added for 45 min. Target cells were washed again to remove nonadherent indicator cells. Titers were defined as the antibody dilution showing 20% positive (rosetted) target cells as evaluated under light microscopy. Indirect immunofluorescence assays were performed as described (8). Cells were fixed in equal volume of methanol/acetone, incubated with serially diluted mouse mAb (see Table I) then with goat anti-mouse immunoglobulins conjugated with fluorescein (Cooper Biomedical, Malvern, PA) at a dilution of 1:40 in PBS. Double-labeling experiments, performed to assess whether individual infected melanocytes expressed both Ia antigens and viral p21 protein, were done as follows: melanocytes were incubated with anti-p21 rat mAb (at a dilution that did not detect endogenous human p21 proteins) followed by goat anti-rat IgG labeled with rhodamine (Cooper Biomedical) diluted 1:25 with PBS. After extensive washing the cells were then incubated with anti-Ia mouse mAb followed by goat anti-mouse IgG labeled with fluorescein (Cooper Biomedical) diluted 1:25 with PBS. To detect expression of MuLV p30 gag protein, fixed cells wre incubated with rabbit anti-Rauscher-MuLV group-specific p30 serum at a dilution of 1:25, washed, then incubated with goat anti-

rabbit IgG labeled with fluorescein (Coopers Biomedical) diluted 1:25 with PBS. Cells were examined with a Nikon fluorescence microscope using either blue or green filters.

Induction Assays. Assays for Ia antigen induction were performed in Falcon 3034 plates (Falcon Labware). Target melanocytes were plated at 200 cells/well in MEM medium plus 7.5% FCS. 2–4 h after plating, spent culture supernatant was added to the medium in each well to give a final dilution of 1:3 (vol/vol). Cells were then cultured at 37°C in 5% CO_2, and serological assays were performed as described above at days 3 and 6 using L243 mouse mAb. Natural IFN-γ was from Interferon Sciences, Inc., New Brunswick, NJ.

Results

Fig. 1 shows the results of Ki-MSV and Ha-MSV infection on the morphology and growth characteristics of human melanocytes. 2 wk after infection, islands of Ki-MSV- or Ha-MSV-infected melanocytes appeared that had a distinct morphology. Uninfected melanocytes grow as bipolar spindle-shaped cells without much intercellular contact (Fig. 1 *A*). Cells infected with the amphotropic MuLV helper virus had the same morphology as normal melanocytes (Fig. 1 *B*). Melanocytes infected with either Ki-MSV or Ha-MSV were more polygonal and grew as clusters of cells with a tendency to pile up (Fig. 1 *C*). Identical changes were observed in four independently Ki-MSV- or Ha-MSV-infected melanocyte cultures. ~90% of melanocytes infected with amphotropic MuLV, Ki-MSV, or Ha-MSV expressed cytoplasmic viral p30 gag gene protein. At 2 wk, 65% of the cells infected with Ki-MSV or Ha-MSV also expressed detectable levels of viral p21 protein. A similar percentage of Ki-MSV- or Ha-MSV-infected cells formed infectious centers on NIH 3T3 cells, indicating the production of mature transforming virus. Supernatants from Ki-MSV- or Ha-MSV-infected cultures contained ~10^2–10^4 infectious focus forming units (FFU) per milliliter as assayed on NIH 3T3 cells. Supernatants from amphotropic MuLV-infected cells produced ~10^4 infectious non–focus forming units per milliliter. Northern blot analysis confirmed the presence of Ha-*ras* or Ki-*ras* specific viral messenger RNAs.

Proliferation of melanocytes in vitro depends upon the addition of exogenous growth factors to the medium (11). Factors that support the growth of melanocytes include TPA (11). and growth factors derived from fetal fibroblasts, astrocytoma and melanoma cells (28). In the absence of growth factors, melanocytes rapidly become senescent and die. In contrast, cultured melanomas grow vigorously in the absence of TPA, suggesting that malignant transformation of melanocytes is associated with the acquisition of autonomy from exogenous growth factors. In the presence of TPA, the growth rate of melanocytes infected with Ki-MSV or Ha-MSV was comparable to uninfected control cells and to a companion culture infected with amphotropic MuLV. In the absence of exogenous growth factors, uninfected melanocytes and melanocytes infected with amphotropic MuLV died rapidly (within 6–12 d). Ki-MSV- and Ha-MSV-infected cultures also become senescent, but at a reduced rate (within 2–4 wk). Thus, expression of *ras* oncogenes does not induce stable, growth factor–independent melanocyte cell lines.

Melanocytes cultured in the presence of TPA do not form colonies in semisolid agar. This is also true of melanocytes infected with amphotropic MuLV. In

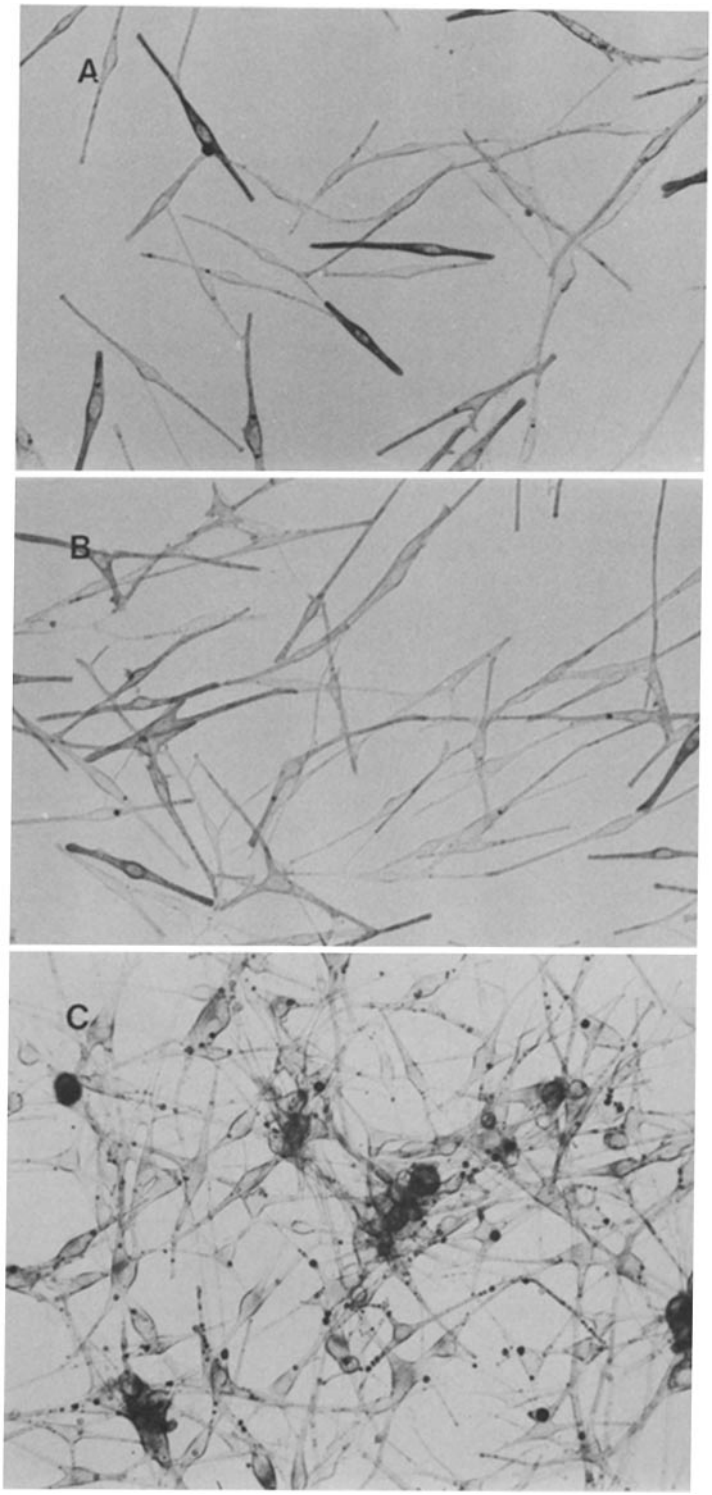

FIGURE 1. Morphology of cultured melanocytes. Photomicrographs of (*A*) uninfected melanocytes, (*B*) 4070A amphotropic MuLV–infected melanocytes, and (*C*) Ki-MSV-infected melanocytes. × 220. Cells shown in *B* and *C* are 3-wk postinfection.

contrast, Ki-MSV- and Ha-MSV-infected melanocytes acquire an anchorage-independent phenotype and initiate colonies of proliferating cells in soft agar assays at a frequency of 0.04–0.1%, but require TPA to do so. We could not determine whether these cells were also tumorigenic in *nu/nu* mice, as these cells release high amounts of infectious MuLV/MSV, which causes leukemias and sarcomas in mice. Melanocytes infected with *ras*-containing viral vectors that do not result in productive infections are being examined to determine if these cells are tumorigenic.

Previous work (9) has shown that melanocytes do not normally express Ia antigens, although Ia expression can be reversibly induced in melanocytes by IFN-γ. A range of other substances were tested for melanocyte Ia-inducing activity, including IFN-α and IFN-β, but only IFN-γ was active (9). Table I shows the results of serological tests for expression of a range of cytoplasmic and cell surface antigens, including class I and class II histocompatibility antigens in infected and noninfected melanocytes. Infection with Ha-MSV or Ki-MSV induced the strong expression of class II antigens in melanocytes within 5–10 d. Induction of Ia antigens was observed in four independently derived melanocyte cultures. Immunological analysis by dual-labeling fluorescence indicated that the cells expressing Ia antigens also expressed viral p21 protein. Ki-MSV- and Ha-MSV-infected melanocytes were tested over a period of 6 mo with no change in Ia expression. Class II antigens were not induced in melanocytes infected with amphotropic MuLV. Little or no change was detected in the expression of class I histocompatibility antigens in melanocytes infected with Ki-MSV, Ha-MSV, or amphotropic MuLV. To determine whether Ia induction by *ras* oncogenes was a reversible phenomenon, as in IFN-γ-treated melanocytes, or a permanent one, as in melanomas, we studied melanocytes infected with a ts mutant of Ki-MSV (14). Fig. 2 shows that both the α (34 kD) and β (28 kD) chains of the Ia bimolecular complex were expressed in equivalent amounts at both permissive and nonpermissive temperatures, suggesting that once Ia antigen expression has been established, continued expression is not dependent on functional viral *ras* p21. It is, of course, possible that minimal amounts of functional p21 exist at the nonpermissive temperature, which may be sufficient to maintain the inductive effect on Ia antigens.

To determine whether Ia expression can be induced in other cell types, early-passage human fetal foreskin fibroblasts and early-passage SK-MEL-93 (DX-2, an Ia^- melanoma [29]) cells were infected with Ki-MSV. No Ia antigen induction was induced in these cells even though 90% of the cells were producing Ki-MSV p21 and infectious transforming virus. Both cell types (whether infected with Ki-MSV or not) expressed Ia after induction by IFN-γ.

To determine which class II subloci were activated, cytoplasmic RNA was analyzed by northern blotting with class II probes. Ki-MSV-infected melanocytes expressed RNA specific for at least three class II α gene loci, DP, DQ, and DR. (see Fig. 3); uninfected melanocytes and melanocytes infected with amphotropic MuLV did not express Ia antigens or RNA specific for class II genes. Immunoprecipitation analysis (Fig. 4) with extracts of metabolically labeled cells showed similar patterns of class I and II antigen expression in melanoma cells and Ki-MSV-infected melanocytes.

TABLE I

Phenotypic Characteristics of Melanocytes and Melanocytes Infected with Retroviruses

Melanocytes (1346)	Serological analysis of antigen expression: reciprocal of serum titers ($\times 10^2$)*									Tyrosinase‡	Pigmentation§	Transforming activity‖	
	Histocompatibility antigens		Melanocyte antigens			Other neuroectodermal antigens							
	HLA-A, B, C	Ia	C350	CF21	TA99	G_{D3}	B2.6	B5.2	MEL-1			FFU	CFU
Uninfected	6,250	—	3,125	16	256	0.1	625	3,125	2.5	42.3	+	0	10^{-4}
Amphotropic	6,250	—	3,125	16	256	0.1	625	3,125	2.5	22.3	+	0	10^{-4}
Ha-MSV	6,250	3,125	3,125	64	64	25	25	3,125	2.5	ND	+	3×10^4	0.04–0.1
Ki-MSV	6,250	3,125	3,125	16	256	25	125	3,125	2.5	39.2	+	3×10^5	0.04–0.1

* Cells were scored for cell surface antigen expression by erythrocyte rosetting assays: HLA-A, B, C, Ia, C350, GD3, B2.6, B5.2, and MEL-1; or for cytoplasmic antigen expression by indirect immunofluorescence: CF21 and TA99. Serum titers represent dilution at which 20% of the cells were positive (rosetted).

‡ Tyrosinase assays were performed as described by Pomerantz (47), as modified by Houghton et al. (3). Tyrosinase activity is expressed as cpm $\times 10^3$ of tritiated H_2O produced per milligram of protein. Nonpigmented renal carcinoma cells (standard) produced a background of 10^3 cpm of ^3H_2O per milligram of protein.

§ Pigmentation was estimated visually by the intensity of brown or black pigment in the cell pellet.

‖ Transforming activity of virus produced by infected cells was determined by focus assays on NIH 3T3 cells. FFU, focus forming units per milliliter of supernatant fluid. For CFU, data shown are the percentages of cells plated per dish which gave colonies of at least 50 cells in 3–4 wk. 10^5 and 5×10^5 cells were plated in 100-mm dishes in 0.3% agar onto a bottom layer of 0.75% agar in quadruplicate.

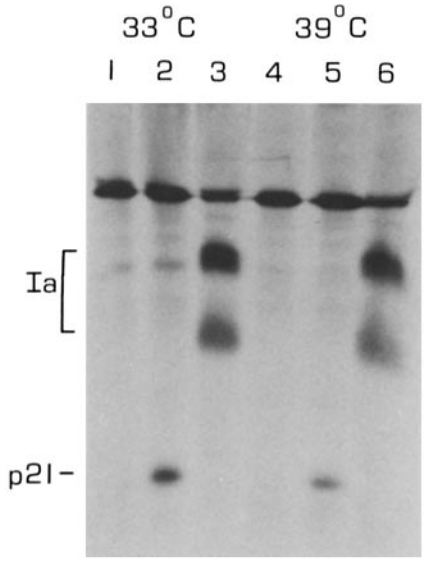

FIGURE 2. Immunoprecipitation of Ia antigens and viral p21 protein. Autoradiograms of immunoprecipitates obtained with extracts of [^{35}S]methionine-labeled melanocytes infected with ts mutant of Ki-MSV and grown at 33°C or 39°C for 8 wk before analysis by SDS-PAGE. Lanes *1*, *4*: unrelated mouse mAb; lanes *2*, *5*: Y13–259 mAb (anti-p21); lanes 3, 6: L243 mAb (anti-Ia).

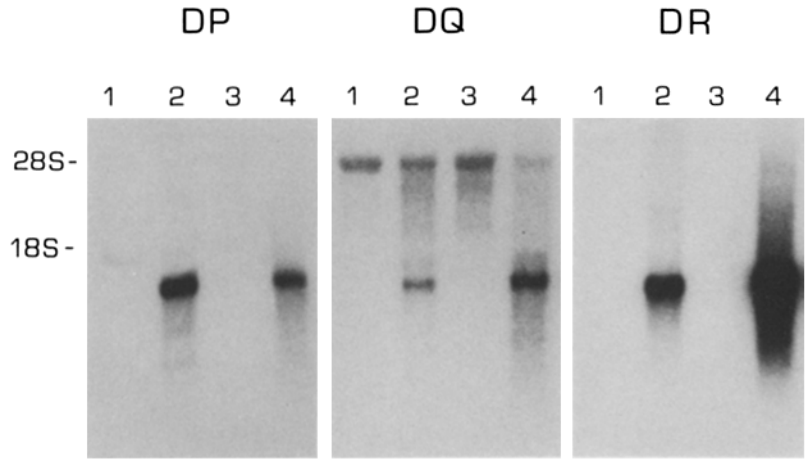

FIGURE 3. Northern blot analysis of class II gene expression. Blots were hybridized to the following ^{32}P-labeled DNA fragments of between 500 and 2,500 nucleotides in length, isolated as described (43). DP-α (pDS-α3,8; a gift of H. Erlich, Cetus Corp., Emeryville, CA); DQ-α (pDQ10-8 [ref. 44]); and DR-α (pDRH2 [refs. 45, 46]). Positions of the 28 S and 18 S ribosomal RNA are indicated. Lanes *1*: uninfected melanocytes; *2*: Ki-MSV-infected melanocytes; *3*: 4070A amphotropic helper MuLV–infected melanocytes; and *4*: SK-MEL-147 melanoma.

Although T lymphocytes are the only cell type known to produce IFN-γ, we considered the possibility that melanocytes synthesize IFN-γ after infection with Ki-MSV or Ha-MSV. A sensitive Ia-induction assay, detecting as little as 1–5 U/ml of IFN-γ (9), was used to assay culture supernatants of Ki-MSV-infected melanocytes. Control and infected melanocytes were cultured for 4 d, and

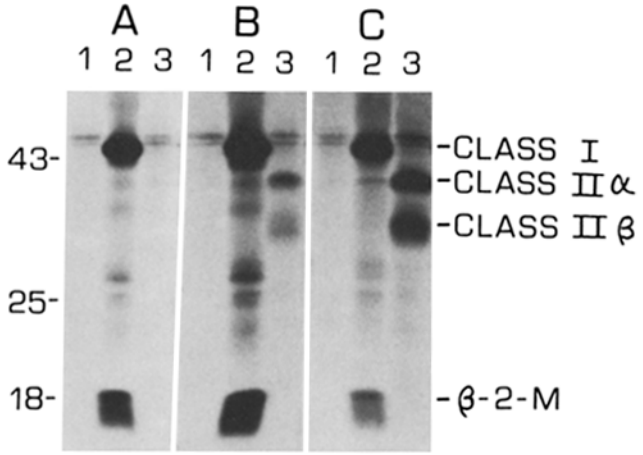

FIGURE 4. Immunoprecipitation of Ia and HLA-A,B,C antigens. Autoradiograms of immunoprecipitates obtained with extracts of the indicated cells labeled with [^{35}S]methionine for 16 h and analyzed by SDS-PAGE. *A*, uninfected melanocytes; *B*, Ki-MSV-infected melanocytes; *C*, SK-MEL-47 melanoma. B-2-M, B-2 microglobulin Lanes: *1*, unrelated mouse mAb (control); *2*, W6/32 mAb (anti-HLA-A,B,C); *3*, L243 mAb (anti-Ia). Molecular mass standards: ovalbumin, 43 kD; α-chymotrypsinogen, 25 kD; β-lactoglobulin, 18 kD.

supernatants were collected, centrifuged to remove virus particles, then added to target melanocytes. Supernatants from uninfected melanocytes to which IFN-γ was added (to final concentrations of 5 and 50 U/ml) served as an additional control for these experiments. Target cells were assayed for the expression of Ia antigens by mixed hemadsorption assays after 3 and 6 d of incubation with supernatants. Supernatants from Ki-MSV-infected melanocytes or uninfected control melanocytes were unable to induce expression of Ia antigens, whereas supernatants to which IFN-γ had been added induced Ia expression. Therefore, we have no evidence for the production of IFN-γ or other Ia-inducing factors by Ki-MSV infected melanocytes.

Melanocytes and melanomas have a number of distinguishing characteristics. In some cases, these are restricted to melanocytes and melanomas (3, 8, 22); in other cases, they are shared by other cell types of neuroectodermal origin (8, 30–32). Tests of Ki-MSV- and Ha-MSV-infected melanocytes for the expression of these differentiation-related phenotypic characteristics are shown in Table I. There was essentially no change in differentiation-related characteristics upon infection with either amphotropic MuLV or *ras* oncogene–containing viruses. Infected melanocytes (*a*) remained pigmented, indicating the presence of melanin; (*b*) had tyrosinase levels that were comparable to uninfected cells; (*c*) expressed melanocytic markers TA99 and CF21, two antigens characteristic of mature melanosomes (22, 23); (*d*) expressed a cell surface glycoprotein, detected by mAb C350, found only on melanocytes and pigmented melanoma cells (A. N. Houghton, unpublished data); and (*e*) continued to express adenosine deaminase binding protein (ADA BP), which is not expressed by melanoma cells (A. N. Houghton, unpublished data). One well-characterized melanoma marker whose expression did change was G_{D3} disialoganglioside. While G_{D3} is expressed at low levels on the surface of melanocytes, it is a predominant membrane

ganglioside of melanomas (8, 24, 33). Melanocytes infected with Ki-MSV showed a substantial increase in the expression of G_{D3} as measured by immune-rosetting assays. Direct quantitation of G_{D3} expression using ^{125}I-labeled antibody indicated that the level of G_{D3} in Ki-MSV-infected melanocytes increased by >5–10-fold over uninfected melanocytes or melanocytes infected with amphotropic MuLV.

Discussion

The constitutive expression of Ia antigens in melanoma, in contrast to the lack of Ia expression by melanocytes, can be accounted for in two ways. Ia expression could be a characteristic of an unidentified early progenitor in the melanocyte pathway that is a target for melanocyte transformation; expression of Ia by melanoma cells would simply reflect their origin from an Ia^+ normal cell population. This idea comes from the finding of Houghton et al. (3) that cultured melanomas from different individuals can be classified into three general groups according to antigenic and morphological features, and that these characteristics appear to correspond to different stages in melanocyte differentiation. The other possibility is that melanomas arise from Ia^- precursors and that induction of Ia expression is one of the consequences of malignant transformation. The present study provides support for this possibility, i.e., rapid Ia induction after Ha-MSV or Ki-MSV infection. Once Ia is induced, however, the presence of functional p21 transforming protein does not seem to be essential for the continued expression of Ia, as indicated by the results with ts viral *ras* mutants. The induction of Ia expression in melanocytes by IFN-γ and viral *ras* appears to have a high degree of specificity. With regard to soluble factors, only IFN-γ has Ia-inducing activity (9). Using amphotropic MuLV constructs with other oncogenes, we have not found Ia in melanocytes expressing *myc*, *src*, or *fms*, emphasizing the unusual capacity of viral *ras* to activate Ia expression. There are several indications that Ia induction by activated *ras* and by IFN-γ are mediated through separate pathways: (*a*) Ia induction by viral *ras* does not appear to involve IFN-γ, (*b*) Ia^- melanomas that are inducible by IFN-γ remain Ia^- after introducing viral *ras*, and (*c*) fibroblasts expressing viral *ras* do not express Ia even though they do so after IFN-γ. At least two *trans*-acting factors regulating class II gene expression in lymphoid cells have been proposed (34, 35). Both factors are necessary for expression of three class II subloci, DP, DQ, and DR. In melanocytes, activated *ras* genes and IFN-γ induce products of these three loci in a coordinate fashion, excluding the possibility that differential activation of class II genes is the basis for differences in Ia induction by IFN-γ and viral *ras*.

Normal diploid human cells, in contrast to rodent cells, have been difficult to transform with activated *ras* oncogenes as the sole transforming agent (36, 37). In no instance have such cells formed tumors in *nu/nu* mice. However, in systems where cells have either a genetically determined chromosomal abnormality, as in Bloom's syndrome (38), or have undergone extensive aneuploidy as a consequence of prolonged passage in tissue culture (39), introduction of an activated *ras* oncogene can induce the full range of transformation characteristics. Our experience with melanocytes is similar to experience with other diploid human cell types. Melanocytes infected with *ras*-containing retroviruses show some characteristics of melanoma, i.e., Ia expression, augmented G_{D3} levels, and ability

to form colonies in soft agar, but not other melanoma characteristics, i.e., growth factor independence and loss of adenosine deaminase binding protein. In addition, melanomas are known to have marked karyotypic abnormalities (40), with the most common perturbations being hyperdiploid to tetradiploid numbers of chromosomes, chromosomal rearrangements, nonrandom alterations on chromosomes 1, 6, and 7, and a frequent loss of heterozygosity at loci on many chromosomes (41), Melanocytes expressing viral *ras* maintain a diploid karyotype during early passage. However, by 8–10 wk after infection, some 50% showed hyperdiploid chromosomal numbers, although no chromosomal rearrangements were observed. In contrast, amphotropic MuLV-infected melanocytes retain a normal karyotype, and cultured melanocytes are known to remain euploid for at least 18 mo (42). It will be important to define the progressive chromosomal changes that occur in viral *ras*-containing melanocytes with prolonged passage, and to determine whether such changes lead to the fully transformed features of melanoma.

Summary

Human melanocytes infected with Ki-MSV or Ha-MSV, but not amphotropic MuLV, undergo a series of transformation-related changes that are characteristic of malignant melanoma. These are (*a*) expression of Ia antigens, in particular DP, DQ, and DR class II histocompatibility gene products, (*b*) a transformed morphology and ability to grow in soft agar, and (*c*) a 5–10-fold increase in the cell surface expression of G_{D3} ganglioside. However, other characteristics of melanoma, such as independence from specific growth factors and loss of adenosine deaminase binding protein were not observed. We conclude that viral *ras* oncogenes initiate early transformation events in melanocytes, and that Ia antigen expression is a transformation marker in this system.

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