SURFACE ANTIGENS OF MELANOMA AND MELANOCYTES

Specificity of Induction of Ia Antigens by Human γ-Interferon

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Although Ia or Class II major histocompatibility antigens have generally been associated with cells of the immune system, recent studies have demonstrated the presence of Ia antigens on cells with no known immune functions (1–8). One of these cell types is malignant melanoma (1, 3). While Ia can be demonstrated on a proportion of both cultured and noncultured specimens of melanoma (9, 10), no Ia antigens have been detected on normal melanocytes, either in vitro (9) or in vivo (10). A possible explanation for this finding is that Ia antigens are expressed on an early, as yet unidentified cell in the normal melanocyte lineage and that Ia^+ melanomas arise from this Ia^+ progenitor (9). According to this view, Ia antigens would be classified as differentiation antigens in the melanocyte pathway, defined as such because they are expressed during an early stage of normal melanocyte differentiation. An alternative view is that the expression of Ia antigens on melanomas is a consequence of events occurring during malignant transformation (3, 9). As a corollary to this view, Ia antigens would not be expressed during normal melanocyte differentiation.

A number of reports have demonstrated that γ-interferon (IFN-γ)[1] can induce or augment the expression of Ia antigens on several different cell types, including melanoma (11, 12). To pursue this observation with regard to normal and transformed cells belonging to the melanocyte series, we have asked the following questions: (*a*) Is induced/augmented expression of Ia antigens by IFN-γ a general feature of melanomas or is it a characteristic of only a subset of melanomas? (*b*) Can normal melanocytes be induced to express Ia? (*c*) How specific is Ia induction by IFN-γ: what is the activity of other species of interferon or other factors known to influence growth or differentiation? (*d*) Is Ia induction part of a coordinate change in the program of cell surface differentiation antigens expressed by melanomas or is it restricted to surface antigens coded for by the major histocompatibility complex, and finally, (*e*) how broad is the range of cell types that can be induced to express Ia by IFN-γ?

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[1] *Abbreviations used in this paper:* FBS, fetal bovine serum; IFN, interferon; MEM, minimal essential medium; MHC, major histocompatibility complex; PA, protein A; PMA, phorbol-12-myristate-β-acetate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCGF, T cell growth factor; β_2m, β_2 microglobulin.

Materials and Methods

Cell Lines. Melanocytes and keratinocytes were cultured as previously described (13, 14). The melanocyte-3 cell line was kindly provided by Dr. M. Eisinger, Sloan-Kettering Institute. Other cell lines were obtained from our cell bank. Cell lines were cultured in Eagle's Minimum Essential Medium (MEM), 2 mM glutamine, 1% nonessential amino acids, 100 U/ml penicillin, and 100 μg/ml streptomycin (MEM medium) supplemented with 7.5% fetal bovine serum (FBS). Cultures were regularly tested for mycoplasma and contaminated cultures were discarded.

Sources of Human IFN and Other Agents. Recombinant IFN-γ was provided by Genentech, Inc. (S. San Francisco, CA) and natural IFN-γ by Interferon Sciences, Inc. (New Brunswick, NJ). The activity of IFN-γ preparations, determined in NIH units by Dr. B. Rubin, Sloan-Kettering Institute, was 1×10^5 U/ml for natural IFN-γ and 2×10^4 U/ml for recombinant IFN-γ. Natural IFN-β 1.5×10^6 U/ml was obtained from Roswell Park Memorial Institute (Buffalo, NY), natural leukocyte IFN-α (Le) 3.3×10^6 U/ml from the New York Blood Center (New York, NY), natural lymphoblastoid IFN-α (Ly) 5.4×10^6 U/ml from the Wellcome Foundation (London, Great-Britain), and recombinant IFN-α 3.4×10^6 U/ml from Hoffmann-LaRoche (Nutley, NJ). Other agents came from the following sources: endotoxin (Novo Pyrexal), Hermal-Chemie Kurt Hermann (Hamburg, Federal Republic of Germany); phorbol 12-myristate-13-acetate (PMA), P-L Biochemicals (Div. of Pharmacia, Inc., Piscataway, NJ); cholera toxin, Schwarz-Mann (Div. of Becton, Dickinson and Co.); retinoic acid, dibutryl cAMP, theophylline, 5-azacytidine, Sigma Chemical Co. (St. Louis, MO); human T cell growth factor (TCGF), glycyl-histidyl-lysine, nerve growth factor, Collaborative Research, Inc. (Waltham, MA). Mouse tumor necrosis factor (15) was provided as a partially purified preparation (20,000 U/mg) by E. Carswell, Sloan-Kettering Institute.

Serological Reagents. The following mouse monoclonal antibodies were used for serological testing:

Monoclonal antibody	Antigenic determinant	Source of antibody or hybridoma (reference)
L243	Ia monomorphic	American Type Culture Collection (ATCC), Rockville, MD (16)
S-1-71	Ia monomorphic	M. Tanimoto, Sloan-Kettering Institute, New York, NY
13-17	Ia monomorphic	(17, 18)
MCS-7	DR3,5,w6	N. Tanigaki, Roswell Park Memorial Institute, Buffalo, NY (19)
BT 3/4	DC-1	G. Corte, Instituto di Chimira Biologica, Genova, Italy (20)
Genex 3.53	DC-1	ATCC (21)
W6/32	HLA-A,B,C	ATCC (22)
BBM.1	β_2m	ATCC (23)
M-1 through M-9, M-13, M-14, M-18, M-19, M-28, M-32, M-34	Melanoma/melanocyte cell surface antigens	(see reference 9)

T87, T43, J143, V1, AJ9, AJ17, AJ425, L101, M368, M138, A123, L2-30	Cell surface antigens expressed by most human cell types, including melanomas and melanocytes	Human Cancer Serology Laboratory, Sloan-Kettering Institute, New York, NY
S4, S22, S25, T138, OM5, J233, JP165, F3, F15, F18	Cell surface antigens expressed by epithelial cell types, but not by melanomas or melanocytes	Human Cancer Serology Laboratory, Sloan-Kettering Institute, New York, NY

Human typing serum Hon (BR4 Class II MHC antigen) has been described previously (24), and the reactivity of natural antibody detecting the Mel-1 differentiation antigen of melanomas and melanocytes (M-10) has been reported (25).

Serological Assays. The anti-mouse immunoglobulin (anti-mouse Ig) and protein A (PA) mixed hemadsorption assays were performed as previously described (26, 27). To prepare indicator cells, anti-mouse Ig (Accurate Chemical and Scientific Corp., Westbury, NY) or PA (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc.) was conjugated to type 0 human erythrocytes using 0.01% chromium chloride. Serological assays were performed on cells previously plated in Falcon 3034 plates. Antibodies were incubated with target cells at 20°C for 1 h. Target cells were then washed and indicator cells added for 1 h. Titers were defined as the antibody dilution showing 50% positive (rosetted) target cells (26).

Induction Assays. Assays for antigen induction were performed in Falcon 3034 plates (Falcon Labware, Div. of Becton, Dickinson and Co., Oxnard, CA). Target cells were plated at 200 cells/well in MEM medium plus 7.5% FBS. 2–4 h after plating, the agent to be tested was added to the medium in each well to give the appropriate final concentration. Cells were then cultured at 37°C in 5% CO_2 and serological assays were performed at indicated intervals.

Metabolic Labeling and Biochemical Identification of Ia Antigens. Cells were labeled with [^{35}S]methionine and the cell extracts were fractionated on a concanavalin A–Sepharose column (Pharmacia, Inc.) and eluted with 0.2 M methyl-α-D-mannoside (18). Eluted extracts were immunoprecipitated (18) and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Results

Induced/Augmented Expression of Ia Antigens by IFN-γ Is a General Feature of Melanoma. A series of melanoma cell lines were tested for expression of Ia antigens before and after exposure to natural IFN-γ or recombinant IFN-γ. Fig. 1 illustrates the response of SK-MEL-113, a melanoma cell line that shows no expression of Ia under normal growth conditions but can be induced to express high levels of Ia after growth in natural or recombinant IFN-γ. Fig. 2 summarizes tests with a panel of 36 melanoma cell lines. In the absence of IFN-γ, 20 melanoma cell lines express Ia in a constitutive fashion and 16 express no Ia. Melanomas with constitutive Ia expression respond to IFN-γ by augmented (*e.g.*, SK-MEL-130), or by little or no change in, Ia expression. In the case of the 16 melanoma cell lines that were non-Ia expressors, 13 could be induced to express Ia by IFN-γ and three remained Ia$^-$. Induced/augmented expression of Ia antigens by IFN-γ was not associated with any changes in cell morphology or pigmentation.

Ia determinants on melanomas having constitutive or induced Ia expression

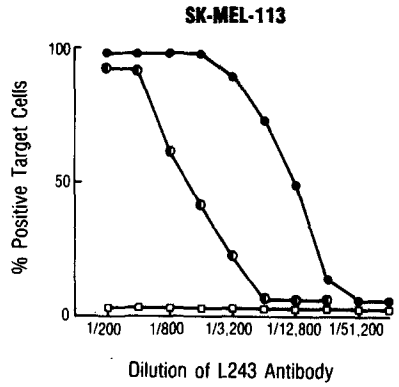

FIGURE 1. Expression of Ia antigens by SK-MEL-113 melanoma cell line after IFN-γ induction. Cells cultured without IFN-γ, □; cells cultured with recombinant IFN-γ 100 U/ml for 3 d, ◒; cells cultured with purified natural IFN-γ 200 U/ml for 3 d, ●. Serological assay: anti-mouse Ig mixed hemadsorption assay with L243 antibody.

could be detected by antibodies directed to monomorphic as well as alloantigenic specificities. Fig. 3 shows tests with two Ia$^-$ melanoma cell lines, SK-MEL-30 and SK-MEL-113, that express Ia after IFN-γ induction. Both monomorphic and alloantigenic Ia determinants were demonstrable.

Induction of Ia expression was related to both the concentration of IFN-γ and the duration of exposure. Preparations of natural IFN-γ and recombinant IFN-γ showed similar inducing activities and were able to induce Ia antigens on melanomas at concentrations as low as 2 U/ml (Fig. 4). Maximum induction of Ia occurred by 3–5 d. When IFN-γ was removed from the culture medium, expression of Ia antigens on SK-MEL-113 cells fell and was undetectable 10 d later (Fig. 5). To demonstrate that this reversal of Ia antigen expression was not due to outgrowth of a subpopulation of cells resistant to induction, melanoma cells were re-exposed to IFN-γ. Upon reinduction, 100% of cells expressed Ia antigens. Thus, expression of Ia antigens did not become constitutive after induction, but rather was reversible and dependent on the continued presence of IFN-γ.

Melanocytes Are Induced to Express Ia Antigens by IFN-γ. In our previous studies, we were unable to detect Ia antigens on cultured melanocytes (9). Fig. 6 shows that exposure of melanocytes to IFN-γ results in high levels of Ia expression. Ia expression was detected by three monoclonal antibodies to Ia monomorphic determinants; in addition, DR and DC-1 determinants could be detected. As observed with melanoma cultures, Ia expression by melanocytes was reversible after induction.

IFN-α, IFN-β and Other Agents Influencing Growth and Differentiation Do Not Induce Melanomas and Melanocytes to Express Ia Antigens. A series of agents including IFN-α and IFN-β were tested for their ability to induce expression of Ia antigens on melanoma cell lines and cultured melanocytes (Table I). Natural IFN-α, recombinant IFN-α, and natural IFN-β did not induce Ia antigens, even up to concentrations of 50,000 U/ml. Both IFN-α and IFN-β were found to increase the expression of HLA,A,B,C and β_2m, and IFN-γ also had this activity (see below). Conditioned media from PHA-stimulated peripheral blood lympho-

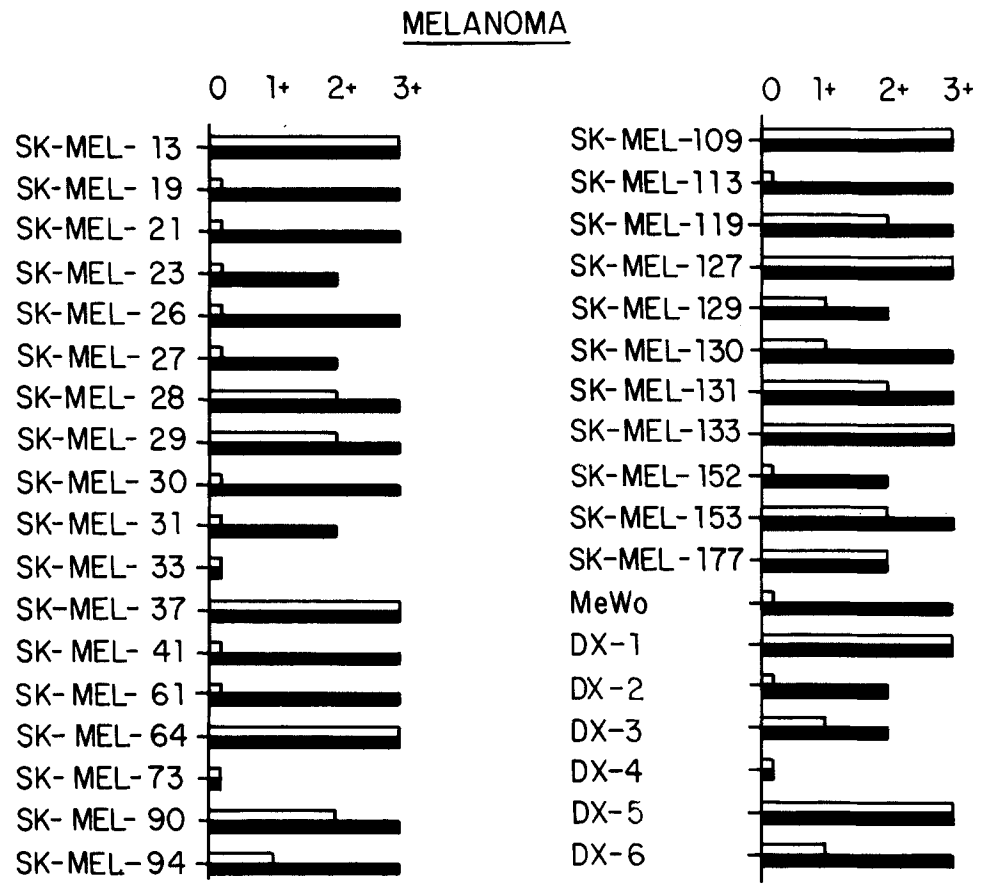

FIGURE 2. Ia phenotype of melanoma cell IFN-γ induction. Cells cultured without IFN-γ, □; cells cultured with natural IFN-γ 200 U/ml for 3 d, ■. Length of horizontal bars represent antibody titers: 0, <1/50; 1+, 1/50—1/1,000; 2+, >1/1,000–1/10,000; 3+, >1/10,000. Serological assay: anti-mouse Ig mixed hemadsorption assay with L243 antibody.

cytes and from mixed lymphocyte cultures were also found to induce Ia antigens, most likely due to the presence of IFN-γ (Table I). A variety of other agents including TCGF, endotoxin, PMA, cholera toxin, retinoic acid, nerve growth factor, 5-azacytidine, tumor necrosis factor, and conditioned media of Ia^+ melanomas did not affect Ia expression.

IFN-γ Does Not Induce or Augment the Expression of Non-HLA-related Antigens on Melanomas and Melanocytes. In contrast to the induced/augmented expression of Ia and HLA,A,B,C antigens, a wide range of other cell surface antigens expressed by melanomas, melanocytes, or other cell types was not influenced by IFN-γ. In these tests, antibodies detecting the following antigenic systems were used (see Materials and Methods): (*a*) 16 melanocyte differentiation antigens, including antigens expressed during early (antigens M-1, M-2, M-3), intermediate (antigens M-4, M-5, M-6) or late (antigens M-9, M-10) stages of melanocyte differentiation (Table II); (*b*) 10 widely distributed antigens that are expressed by most human cell types, and (*c*) 12 antigens that are not found on melanomas

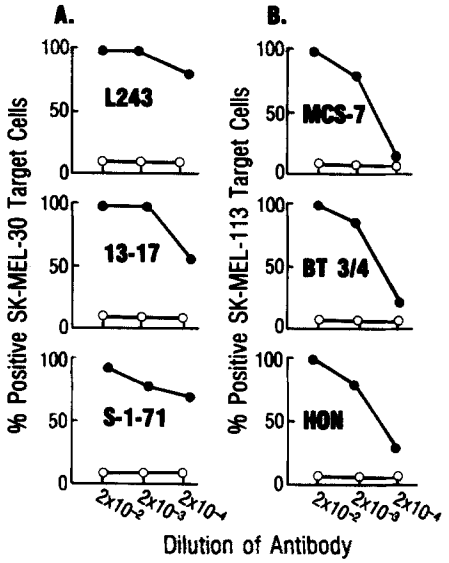

FIGURE 3. Expression of Ia monomorphic and Ia alloantigenic determinants on melanoma cells after IFN-γ induction. (*a*) SK-MEL-30 melanoma cells tested with antibodies to Ia monomorphic determinants (L243, 13-17, S-1-71); (*b*) SK-MEL-113 melanoma cells tested with antibodies to Ia alloantigenic determinants; MCS-7 (anti-DR-3,5,w6), BT 3/4 (anti-DC-1) and Hon (anti-BR4X7). Cells cultured without IFN-γ, O; cells cultured with purified natural IFN-γ 200 U/ml for 3 d, ●. Serological assay: anti-mouse Ig mixed hemadsorption with L243, 13-17, S-1-71, MCS-7 and BT 3/4 antibodies and protein A assay with antibody Hon.

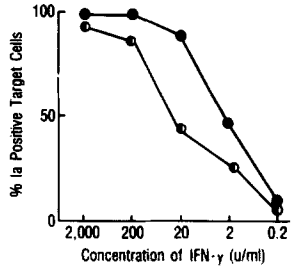

FIGURE 4. Expression of Ia antigens by SK-MEL-113 cells after induction with different concentrations of IFN-γ. Cells cultured with recombinant IFN-γ for 3 d, ◐; cells cultured with purified natural IFN-γ for 3 d, ●. Serological assay: anti-mouse Ig mixed hemadsorption assay with L243 antibody (diluted 1:5,000).

or melanocytes, but are expressed by epithelial cell types (*e.g.*, lung cancer, bladder cancer, and colon cancer). These tests show the striking specificity of IFN-γ for products of the *HLA* (and $\beta_2 m$) loci.

A Broad Range of Cell Types Can Be Induced to Express Ia Antigens by IFN-γ. To determine the range of cell types that can be induced to express Ia by IFN-γ, a panel of human cancer cell lines and cultured normal cells representing a number of distinct differentiation lineages were selected for study (Fig. 7). Constitutive expression of Ia was frequent in astrocytoma cell lines (7/19 Ia^+). However, in contrast to melanoma and astrocytoma, constitutive Ia expression was an uncommon phenotype of epithelial cell types, teratocarinomas, choriocarcinomas, and

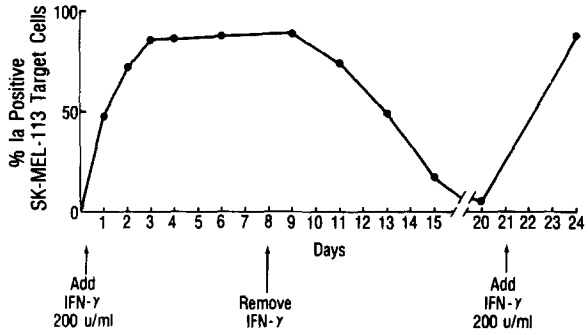

FIGURE 5. Expression of Ia antigens by SK-MEL-113 cells after IFN-γ induction: effect of IFN-γ removal and reinduction with IFN-γ. Cells were cultured in the presence of purified natural IFN-γ 200 U/ml for 8 d. IFN-γ was removed on day eight and re-added on day 21. Serological assay: anti-mouse Ig mixed hemadsorption assay with L243 antibody (diluted 1:5,000).

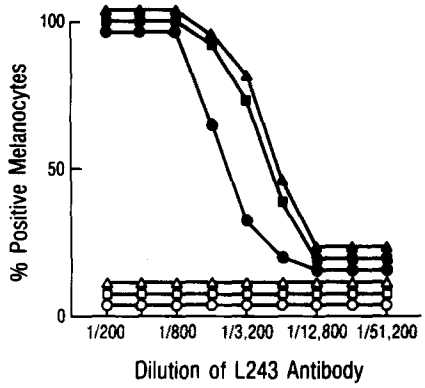

FIGURE 6. Expression of Ia antigens by melanocytes after induction with purified natural IFN-γ 200 U/ml for 3 days. Melanocytes-1 uninduced (○) and induced (●); melanocytes-2 uninduced (□) and induced (■); melanocytes-3 uninduced (△) and induced (▲). Serological assay: anti-mouse Ig mixed hemadsorption assay with L243 antibody.

neuroblastomas. Many of the epithelial cell lines could be induced to express Ia by IFN-γ. Teratocarcinoma, choriocarcinoma, and neuroblastoma were exceptions in this regard; only 1 of 16 cell lines derived from these tumor types showed weak Ia expression after exposure to IFN-γ.

Induction of Ia antigens was confirmed by immunoprecipitation analysis of metabolically labeled cells. In the example shown in Fig. 8, both heavy and light chains of the Ia molecule were detected in extracts of the melanoma cell line SK-MEL-113 and the colon cancer cell line HT-29 after induction with IFN-γ, but not after induction with IFN-α. No Ia antigen was detected in extracts of choriocarcinoma cell line GCC-SV(C) before or after IFN-γ induction.

Parallel tests for expression of HLA-A,B,C products and β_2m indicated that IFN-γ could induce the expression of these antigens in cell types that were noninducible for Ia expression. Fig. 9 illustrates the responses of three cell types to IFN-γ with regard to Ia and HLA-A,B,C expression. SK-MEL-37 has a constitutive Ia^+/HLA-A,B,C^+ phenotype that is not altered by IFN-γ. Melano-

TABLE I

Assays for Induction of Ia Antigen Expression: Summary of Results with Melanoma SK-MEL-113 and Melanocytes

Agent	Concentrations tested	Ia induction
IFN-γ, natural	0.5 U/ml–5×10^4 U/ml	+
IFN-γ, recombinant	0.5 U/ml–5×10^4 U/ml	+
IFN-β, natural	50 U/ml–5×10^4 U/ml	–
IFN-α, Le	50 U/ml–5×10^4 U/ml	–
IFN-α, Ly	50 U/ml–5×10^4 U/ml	–
IFN-α, recombinant	50 U/ml–5×10^4 U/ml	–
Endotoxin	10^{-8} g/ml–10^{-10} g/ml	–
PMA	10^{-8} g/ml–10^{-10} g/ml	–
Cholera toxin	10^{-8} M–10^{-10} M	–
Retinoic acid	10^{-5} M–10^{-8} M	–
Nerve growth factor	10^{-7} g/ml–10^{-9} g/ml	–
Dibutryl cAMP	10^{-2} M–10^{-4} M	–
Theophylline	10^{-3} M–10^{-4} M	–
5-Azacytidine	5×10^{-7} M–5×10^{-8} M	–
Glycyl-histidyl-lysine	10^{-6} g/ml–10^{-7} g/ml	–
Mouse tumor necrosis factor	1 μg/ml–30 μg/ml	–
Human TCGF	5–20 half-max. U/ml	–
Phytohemagglutinin	5 μg/ml	–
Conditioned medium from:		
Ia$^+$ melanomas	½ dil–1/10 dil	–
PHA-stimulated PBL	½ dil–1/10 dil	+
Mixed lymphocyte reactions	½ dil–1/10 dil	+

Cells were cultured for 5 d with a range of concentrations of inducing agents and tested for Ia antigen expression in the anti-mouse Ig mixed hemadsorption assay using L243 antibody.

cytes have an Ia$^-$/HLA-A,B,C$^+$ phenotype before IFN-γ induction, and convert to Ia$^+$ after induction (with little or no change in HLA-A,B,C expression). Tera-2 lacks demonstrable Ia or HLA-A,B,C expression when grown in the absence of IFN-γ. IFN-γ induces HLA-A,B,C expression in Tera-2 cells, but does not induce Ia expression. Three other teratocarcinomas (Tera-1, 577M, 833K), five neuroblastomas (LA-N-In, SH-EP-15, SMS-KAN, SMS-MSNb, SMS-SAN), and one choriocarcinoma [GCC-SV(C)] have also shown this dissociation between Ia and HLA-A,B,C induction by IFN-γ.

Discussion

Established melanoma cell lines show wide variation in Ia expression, from lines with uniformly strong Ia expression to those that show no Ia expression. Melanomas with constitutive expression of Ia respond to IFN-γ with augmented or with little or no increase in Ia expression, whereas non-Ia expressors fall into two groups on the basis of Ia induction by IFN-γ: inducible (13 lines) or noninducible (3 lines). IFN-γ has remarkable specificity with regard to its effect on Ia expression by melanoma; IFN-α and IFN-β do not have this action and 12 other agents with growth/regulatory activity for cells were also without effect. Equally striking is the fact that the inducing effect of IFN-γ was restricted to Ia

TABLE II

Influence of IFN-γ on the Cell Surface Phenotype of Melanoma SK-MEL-113 and Melanocytes: Summary of Results with Monoclonal Antibodies Detecting 19 Different Surface Antigen Systems

Cell surface antigen	SK-MEL-113		Melanocyte-1	
	Titer −IFN-γ	Titer + IFN-γ*	Titer −IFN-γ	Titer + IFN-γ*
Ia	—	5×10^{-5}	—	2×10^{-4}
HLA-A,B,C	5×10^{-4}	1.25×10^{-4}	3.3×10^{-4}	1×10^{-4}
β_2m	1×10^{-3}	2.5×10^{-4}	2.5×10^{-4}	1×10^{-4}
M-1	—	—	—	—
M-2	—	—	—	—
M-3	—	—	—	—
M-4	10^{-5}	10^{-5}	10^{-4}	10^{-4}
M-5	10^{-4}	10^{-4}	10^{-4}	10^{-4}
M-6	10^{-4}	10^{-4}	10^{-4}	10^{-4}
M-7	10^{-3}	10^{-3}	10^{-3}	10^{-3}
M-8	10^{-5}	10^{-5}	10^{-5}	10^{-5}
M-9	10^{-3}	10^{-3}	10^{-3}	10^{-3}
M-10	10^{-2}	10^{-2}	10^{-2}	10^{-2}
M-14	10^{-3}	10^{-3}	10^{-5}	10^{-5}
M-18	10^{-4}	10^{-4}	10^{-3}	10^{-3}
M-19	10^{-5}	10^{-5}	10^{-4}	10^{-4}
M-28	10^{-5}	10^{-5}	10^{-5}	10^{-5}
M-32	10^{-6}	10^{-6}	10^{-6}	10^{-6}
M-34	—	—	10^{-5}	01^{-5}

* Cells were cultured for 5 d with or without purified natural IFN-γ 100 U/ml and tested for antigen expression by anti-mouse Ig mixed hemadsorption or protein A assays. Titer refers to dilution of antibody showing 50% rosetted cells.

and HLA-A,B,C/β_2m expression and did not extend to the expression of a wide range of other cell surface antigens or to other phenotypic traits such as pigmentation or morphology. These findings with melanoma cells and melanocytes indicate that Ia induction by IFN-γ is a result of selective effects involving products of Class I/II MHC genes, rather than the result of a more general change in the differentiation program. Similar studies by others have demonstrated that IFN-γ, but not IFN-α or IFN-β, enhances expression of Ia antigens on the surface of melanoma cells (11, 12). In their investigation of the mechanism of Ia regulation, Rosa et al. (12) showed that HLA-DR mRNA could be increased by IFN-α, IFN-β, and IFN-γ. However, only IFN-γ was found to produce a corresponding increase in the cell surface expression of HLA-DR, and even in this case the increase in HLA-DR mRNA produced by IFN-γ did not always correlate with surface expression of HLA-DR antigen. Thus, IFN-γ appears to regulate Ia antigen expression at the level of transcription, but posttranscriptional events are evidently necessary for expression of Ia at the cell surface.

From our past studies of cell surface antigens of cultured melanomas and melanocytes, we proposed that melanomas could be placed into three categories on the basis of cell surface markers, morphology, and pigmentation, and that

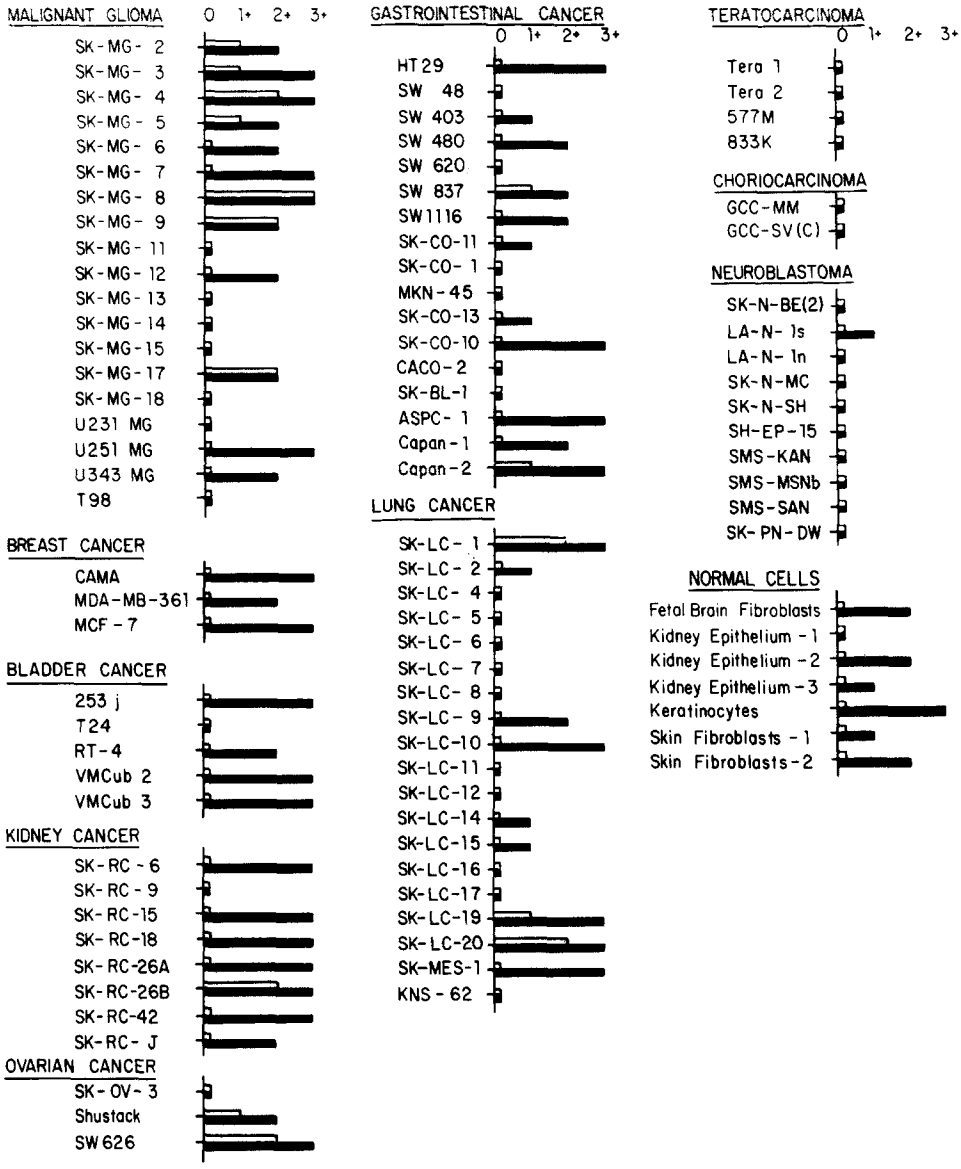

FIGURE 7. Ia phenotype of cultured cell lines before and after IFN-γ induction. Cells cultured without IFN-γ, □; cells cultured with natural IFN-γ 200 U/ml for 3 d, ■. Length of horizontal bars represent antibody titers: 0, <1/50; 1+, 1/50–1/1,000; 2+, >1/1,000–1/10,000; 3+, >1/10,000. Serological assay: anti-mouse Ig mixed hemadsorption assay with L243 antibody.

melanomas in each of these categories mirrored the phenotypic characteristics of normal cells in early, intermediate, or late stages of melanocyte differentiation (9). Because Ia antigens are strongly expressed on melanoma cells corresponding to early or intermediate stages of melanocyte differentiation, with weak or no expression to well-differentiated melanoma cells, we concluded that Ia^+ melanomas correspond to progenitors at an earlier stage in the melanocyte lineage,

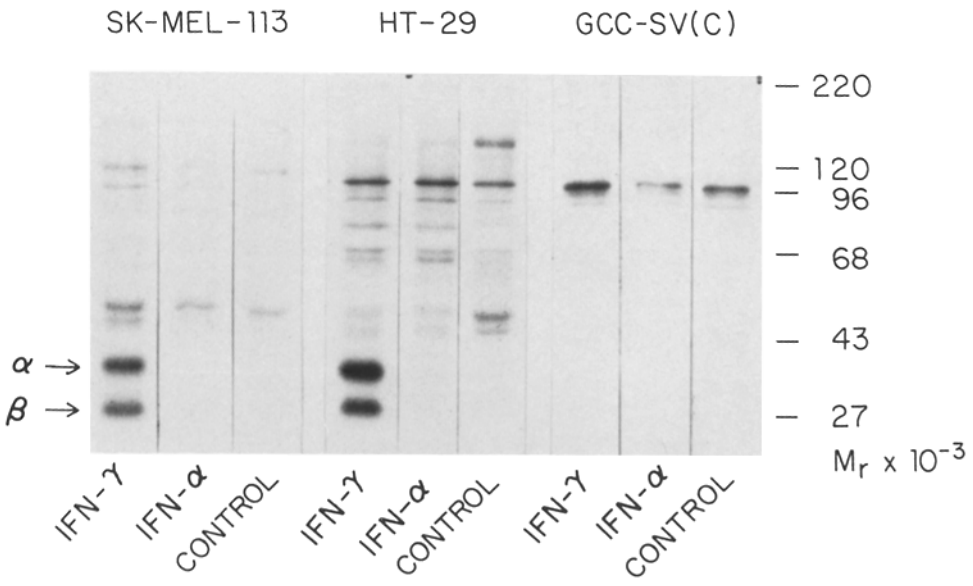

FIGURE 8. Immunoprecipitation of [^{35}S]methionine-labeled extracts of human cell lines SK-MEL-113 (melanoma), HT-29 (colon cancer), and GCC-SV(C) (choriocarcinoma) with L243 antibody. Immunoprecipitates analyzed by SDS-PAGE. Noninduced cells (*CONTROL*), cells induced with purified natural leukocyte IFN-α 500 U/ml for 3 d (IFN-α) and cells induced with purified natural IFN-γ 200 U/ml for 3 d (IFN-γ). α and β refer respectively, to heavy and light chains of Ia molecules.

whereas Ia^- melanomas correspond to a late stage. Normal melanocytes from fetal, newborn, and adult skin do not express Ia antigens and, therefore, were thought to represent later stages in melanocyte differentiation. With the finding that melanocytes, similar to Ia^- melanomas, can be induced to express Ia antigens by IFN-γ, it appears that cells in the melanocyte series are programmed to express Ia antigens, perhaps constitutively during early stages of melanocyte differentiation, and as an inducible trait during later stages. This view depends, of course, on the existence of an Ia^+ melanocyte precursor, and attempts to isolate such cells are underway. An alternative way to interpret the existence of Ia^+ melanomas is that constitutive Ia expression is a transformation-related trait that distinguishes malignant melanocytes from normal melanocytes (9, 10). The closest precedent for this in experimental systems would be the anomalous expression of TL antigens in the TL^+ leukemias arising in TL^- (nonexpressor) mouse strains (28). Further studies of Ia expression during normal melanocyte differentiation and analysis of the structure and regulation of Class II genes in melanoma cells will be necessary to resolve these questions.

It has been a surprise to find that so many different cell types express Ia normally or can be induced to express Ia antigens. In addition to Ia expression by cells involved in immune recognition, Ia antigens have been found on myeloid cells (29), endothelial cells (30, 31), fibroblasts (31), erythroid cells (32), and epithelial cell types (2, 4–8). Our investigation of cultured cell lines suggests that Ia antigen expression can be induced in many, if not most cell types. Similar to our findings with melanoma cell lines, nonmelanoma cell types fall into three

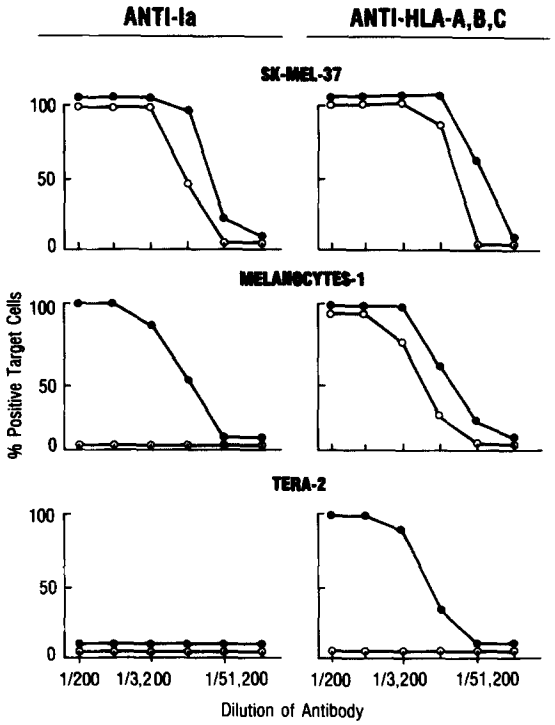

FIGURE 9. Influence of IFN-γ on the expression of Ia and HLA-A,B,C antigens by three human cell types. Cells cultured in the absence of IFN-γ, O; cells cultured with purified natural IFN-γ 200 U/ml for 3 d, ●. Serological assay: anti-mouse Ig mixed hemadsorption assay with L243 antibody (anti-Ia) and W6/32 antibody (anti-HLA-A,B,C).

general categories with regard to Ia expression and response to IFN-γ induction: constitutive, inducible, and noninducible. The distinctive characteristic of the noninducible category is that it includes tumor cell types that arise from germ cells (teratocarcinoma, choriocarcinoma) or correspond to relatively early stages of development (neuroblastoma). HLA-A,B,C antigens and β_2m, which are frequently expressed in low or undetectable amounts in this group of tumors, can be induced by IFN-γ. These findings indicate that the induction of Class I and Class II MHC products by IFN-γ is independently regulated, and that Class I antigens may be susceptible to induction at an early stage of development, while Class II antigens may only be induced in more differentiated cell types. In addition, the Ia noninducible phenotype could have several explanations, ranging from lack of transcription of Class II genes to transcription of Class II genes without subsequent translation or incorporation of the Ia products into the cell surface.

Ia molecules have been found to participate in immune reactions at several levels, from initial antigen recognition to providing signals for immune regulation. The fact that many different cell types not belonging to the immune system express Ia constitutively or can be induced to express Ia by IFN-γ raises the possibility that most somatic cell types can function as antigen-presenting or immune regulatory cells. Alternatively, in addition to their role in the immune

response, Ia antigens could function in a broader, nonimmune context. The appearance of Ia antigens during defined stages in erythroid and myeloid development (32, 33) suggests that Ia antigens have a role in differentiation that is not related to the immune response.

Summary

IFN-γ is known to induce expression of Ia antigens on a variety of cell types. In the present study, this activity of IFN-γ has been analyzed with a panel of 36 melanoma cell lines, normal melanocytes, and 97 cell lines representing a range of other differentiation lineages. 55% of the melanoma cell lines express Ia antigens in a constitutive manner without IFN-γ induction. Of the 16 Ia^- melanoma lines, 13 could be induced to express Ia antigens by IFN-γ, whereas three were noninducible. Melanocytes, which do not normally express Ia antigens, are converted to Ia expression by IFN-γ. Ia antigens expressed constitutively or after IFN-γ induction were identified with antibodies detecting monomorphic and allomorphic products of DR and DC loci. IFN-γ appeared to be unique in its ability to induce Ia expression on melanoma and melanocytes; 14 other agents (including IFN-α and IFN-β) known to influence growth or differentiation did not have Ia-inducing activity. Equally striking is the restriction of antigenic changes following IFN-γ induction to HLA-associated products; of the 38 systems of cell surface antigens examined, only HLA-A,B,C, β_2m, and Ia antigens were affected. A variety of other Ia^- cell types were shown to be Ia-inducible by IFN-γ; these included established lines of breast, colon, pancreas, bladder, kidney, ovary, and brain cancers, and cultures of normal fibroblasts, kidney epithelia, and epidermal keratinocytes. In contrast, three tumor types, teratocarcinoma, choriocarcinoma, and neuroblastoma, were not inducible for Ia expression, even though IFN-γ could induce expression of HLA-A,B,C products. The broad representation of Ia antigens on most somatic cell types expressed either constitutively or after IFN-γ can be viewed in an immunological context (antigen presentation/immune regulatory signals) or could indicate that Ia products have functions other than those related to immune reactions.

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