Classes I and II HLA and Melanoma-associated Antigen Expression and Modulation on Melanoma Cells Isolated from Primary and Metastatic Lesions

Donatella Taramelli, Giuseppe Fossati, Arabella Mazzocchi, Domenico Delia, Soldano Ferrane, and Giorgio Parmiani

Division of Experimental Oncology D [D. T., G. F., A. M., G. P.] and A [D. D.] Istituto Nazionale Tumori, 20133 Milan, Italy, and Department of Microbiology and Immunology, New York Medical College, Valhalla, New York, [S. F.]

ABSTRACT

Human melanoma cells freshly isolated from 20 patients with primary and 73 patients with metastatic melanomas were analyzed by indirect immunofluorescence staining with monoclonal antibodies (MoAb) to class I (HLA-A, -B, and -C) and class II (HLA-DR and -DQ) antigens and to melanoma associated antigen (MAA). The latter included the GD3-MAA and the high molecular weight MAA. HLA class I antigens were present in 91 and 93% of primary and metastatic tumors, respectively. GD3-MAA was detected in 100% of primary and 80% of metastatic tumors. Whereas the high molecular weight MAA was expressed in 75% of tumors. Sixty % of primary and 50% of metastatic melanomas were stained by anti-HLA-DR MoAb, whereas 38 and 21% of cases, respectively, were stained by anti-HLA-DQ MoAb.

Marked phenotypic heterogeneity was evident among primary and metastatic tumors, including different metastases from the same patient. Moreover, in vitro culture of melanoma cells isolated from metastases was associated with an increase from 50 to 75% of tumors stained by anti-HLA-DR MoAb but not of tumors positive for HLA class I antigens and MAA. In vitro incubation with partially purified or recombinant human γ-interferon enhanced the expression of HLA-DR antigens on all short-term cultured melanoma cells tested but induced and/or augmented the expression of HLA-DQ antigens only in 5 of the 8 cases examined. The average increase in antigenic expression was higher for HLA-DQ than for HLA-DR antigens. Flow cytometric measurement of DNA content of melanoma cells treated with γ-interferon revealed that the increase of HLA-DR and -DQ expression induced by γ-interferon was independent from the cell cycle of the tumor cells.

INTRODUCTION

Human malignant melanoma cells have been shown to be heterogeneous with respect to many phenotypic characteristics including the expression of HLA classes I and II antigens and MAA\(^1\) \((1-6)\). Differences in HLA antigens and MAA expression between autologous primary and metastatic melanomas as well as among different metastases from the same patient have been described \((7-9)\). Since both HLA classes I and II antigens may play a crucial role in the interaction of tumor cells with the host's immune response, attempts have been made to correlate the expression of these antigens on melanoma cells with their metastasizing properties, tumor progression, and the patient's immune response. In particular, loss of HLA class I antigens and/or increased expression of HLA class II antigens on human melanomas have been associated with invasiveness and progression of cancer \((10-11)\). Moreover recent work from this laboratory \((12, 13)\) and others \((14)\) has shown that the expression of HLA class II antigens on primary or metastatic melanoma cells is associated with either stimulation or inhibition of autologous antitumor responses in vitro. Therefore it appears that the antigenic phenotype of melanoma cells might be used as a prognostic factor of the in vitro antitumor immune reactivity of the patients.

Most of the studies on the expression of HLA antigens on melanomas have been performed on long-established cell lines or cryostatic sections of surgically removed tumors. To better correlate phenotypic variations with the in vitro immune responses, we have analyzed the expression of HLA class I, class II DR and DQ antigens, and MAA on melanoma cells isolated from fresh tumor samples of primary and metastatic lesions.

Furthermore since it has been reported that lymphokines, namely IFNs, can induce and/or increase the expression of HLA antigens on both normal cells and long-term cultured tumor lines \((15-22)\), we have investigated the susceptibility to modulation by IFNs of HLA on freshly isolated melanoma cells.

Here we report that both primary and metastatic melanomas either freshly explained or cultured in vitro are heterogeneous for the expression of MoAbs-defined HLA classes I and II antigens and MAA. In particular, HLA-DQ antigens appear to be less expressed than HLA-DR on primary and metastatic melanomas. However, no significant differences in the distribution of HLA classes I and II antigens and MAA between primary and metastatic melanomas were observed. Heterogeneity was also evident when the cells were treated with IFNs since differential effects on the expression of HLA class I versus HLA class II antigens were seen in different melanomas. Moreover the modulation of HLA-DQ by treatment with IFN-γ or rHu-IFN-γ seemed...
to occur independently from that of HLA-DR. The phenotypic changes induced by IFN-γ were not cell cycle related since both treated and untreated melanoma cells had a similar fraction of cells in the various phases of the cell cycle as evaluated by the analysis of DNA content.

MATERIALS AND METHODS

Tumor Cells. Fresh tumor cells were isolated from primary cutaneous lesions of 20 patients and from lymph node metastases of 73 patients with malignant melanoma, 1 or 2 h after surgery utilizing the procedure previously described (12). Briefly, tumor specimens were cleaned of necrotic tissues and RBC were removed by hypotonic shock with ammonium chloride. Dead cells were eliminated by treatment of the tumor cell suspension with DNase (270 units/ml) and trypsin (0.25%) (Sigma, Walkersville, MD) supplemented with 10% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, New York, NY) 100 U/ml of penicillin (100 units/ml), streptomycin (100 μg/ml), 15 mm 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid buffer, (Flow Laboratories, McLean, VA) and glutamine. Tumor cell suspensions were then frozen and stored at −80°C or in liquid nitrogen until use. In some cases necrotic tissues and RBC were removed by hypotonic shock with am

viability as assessed by trypan blue exclusion. All tumor cell prepar

25110; Coming Glass Works, New York, NY) trypsinized when confluent of tumor cells. Melanoma cells were cultured in tissue culture flasks (no. 25110; Coming Glass Works, New York, NY) trypsinized when confluent and transferred into new flasks in CM. When needed, tumor cells were mechanically harvested, washed twice with CM, and used in the assays. Both fresh cryopreserved and cultured melanoma cells had at least 80% viability as assessed by trypan blue exclusion. All tumor cells prepara

Normal Cells. Peripheral blood lymphocytes were isolated from hepar

rinized blood of normal donors and of melanoma patients by fractiona

on Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) fol

were then washed and the appropriate concentrations of IFNs were

added in 0.3 ml of CM. After 72–96 h, plates were centrifuged, washed
twice to remove the residual IFNs, and stained by IF, as described above.

RESULTS

Phenotypic Characteristics of Human Melanoma Cells. Table 1 shows the antigenic profile of cell suspensions obtained directly from 20 different primary lesions of malignant melanoma and from 73 metastases. Fifty-four of the latter were tested as freshly isolated cells and 36 after short-term culture in vitro. The latter included cells from 17 melanomas which were analyzed both after isolation and after 2–3 in vitro passages to evaluate directly the effects of in vitro culture on the phenotype of the cells. HLA class I antigens, GD3 MAA, and HMW-MAA were present in a high percentage of both primary and metastatic lesions. HLA-DR antigens were detected on freshly isolated melanoma cells in 60 and 50% of primary and metastatic lesions, respectively. HLA-DQ antigens, however, were found in 38 and 21% of primary and metastatic melanoma lesions. None of these differences between primary and metastatic lesions reached the level of statistical significance. With adaptation in culture a sign

ificant increase in the percentage of HLA-DR and -DQ positive cells stained by anti-HLA and anti-MAA MoAb showed marked

significant increase in the percentage of HLA-DR and -DQ positive cells stained by anti-HLA and anti-MAA MoAb showed marked

Significance. The following mouse MoAb were used: the anti-HLA-ABC monomorphic W6/32 (Sera Laboratories, Sussex, United Kingdom); the anti-HLA-DR monomorphic D1-12 (23) and BT2.9 (24); the anti-HLA-DQ SPV-L3 (25), TU 22 (26), Leu 10 (Becton Dickinson, Sunnyvale, CA); the anti-HLA-D01 BT3.4 (27); the anti HMW-MAA 225.28S (28); and the anti-GD3 MAA R24 (29). Fluorescein isothiocyanate-F(ab')2 fragments of sheep anti-mouse immunoglobulin antibodies (NEI 504) were purchased from New England Nuclear (Boston, MA).

Fluorescence Analysis. IIF staining was performed by incubating 2–6 × 106 cells with the appropriate concentration of the first antibody for 20 min at room temperature in 0.1 ml of CM in flat-bottomed 96-well microtiter plates (Costar 3596; Costar, Cambridge, MA). Cells were then washed three times with CM and resuspended in 0.1 ml of fluorescein-conjugated F(ab')2 fragments of sheep anti-mouse immunoglobulin antibodies (NEI 504; New England Nuclear) for 20 min on ice. Cells were then washed three times, resuspended in 0.1 ml of 1% paraformaldehyde in CM for storage, and analyzed by flow cytometry (FACS IV; Becton Dickinson) or by UV microscope. Results are expressed as the percentage of fluorescent cells above the background fluorescence determined using the same procedure without the primary antibody. A percentage of fluorescent cells above 10 was considered as positive. For each sample, mean levels of fluorescence intensity were calculated from the FACS histograms and expressed in arbitrary units.

For DNA analysis, the cells were fixed in 70% ethanol for 20 min and then stained with chromomycin A3 (Sigma) as described by Johannisson and Thorell (30). The DNA content of each cell was evaluated by FACS analysis using a 455-nm laser excitation wavelength. The percentage of cells occupying S-G1 and M stages of the cell cycle over the total number of cells was calculated and reported.

In Vitro Treatment with IFNs. The following preparations of human IFNs were used: (a) partially purified human IFN-γ (specific activity, 3.4 × 104 units/mg of protein; Meloy Laboratories, Springfield, VA); and (b) rHuIFN-γ 0584 and rIFN-α A/D, kindly supplied by Hoffman La Roche (Nutley, NJ). Treatment with IFNs was performed as previously described (13). Briefly tumor cell cultures were trypsinized and transferred into small culture flasks (Corning Glass No. 25100) at 106 cells/flask in 15 ml CM. After 24 h of culture, CM was discarded and the monolayer cultures were further incubated in 10 ml CM or with the addition of the appropriate concentration of IFN. After 72–96 h, tumor cells were me

chanically harvested and phenotypically monitored by IIF. Alternatively tumor cells, normal lymphocytes, or normal adherent cells were incubated at 1–2 × 105 cells/well in 96-well microtiter plates in CM overnight. Plates were then washed and the appropriate concentrations of IFNs were added in 0.3 ml of CM. After 72–96 h, plates were centrifuged, washed
twice to remove the residual IFNs, and stained by IF, as described above.
HLA AND MAA ON PRIMARY AND METASTATIC MELANOMAS

Table 1

Phenotypic characteristics of human melanoma cells isolated from primary and metastatic lesions

All tumors were examined by IIF using MoAb and fluorescein isothiocyanate-conjugated sheep F(ab')2 anti-mouse immunoglobulin antibodies (see "Materials and Methods" for details).

<table>
<thead>
<tr>
<th>No. of positive cases/no. of cases examined for</th>
<th>Class II</th>
<th>MAA</th>
</tr>
</thead>
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<tr>
<td></td>
<td>DR</td>
<td>DQ</td>
</tr>
<tr>
<td>Primary lesions</td>
<td>12/20 (60)</td>
<td>5/13 (38)</td>
</tr>
<tr>
<td>Metastases</td>
<td>27/54 (50)</td>
<td>4/19 (21)</td>
</tr>
</tbody>
</table>

Table 2

Antigenic changes associated with in vitro culture of melanoma cells isolated from metastatic lesions

All tumors were examined by IIF using MoAb and fluorescein isothiocyanate-conjugated sheep F(ab')2 fragments of anti-mouse immunoglobulin antibodies (see "Materials and Methods" for details).

Table 3

Antigenic heterogeneity of melanoma cells isolated from autologous metastases

Tumor cells were stained with MoAb W6/32, D1-12, SPV-L3 and R24 (anti-GD3 and MAA), respectively, and fluorescein isothiocyanate-conjugated F(ab')2 sheep anti-mouse immunoglobulin antibodies.

Chart 1. Phenotypic heterogeneity of human melanoma cells isolated from primary and metastatic lesions. All the tumors examined were divided into the 3 following groups according to percentage of fluorescence-positive cells: <10% (I); 11-50% (II); and >50% (III). Tumor cells were analyzed by IIF using MoAb and fluorescein isothiocyanate-conjugated F(ab')2 sheep anti-mouse immunoglobulin antibodies (see "Materials and Methods" for details). Pr, primary melanoma; Met, metastatic melanoma; numbers, number of cases with a given phenotype.

DR positive cells, 6 cases high levels, and 8 low levels. Of the 54 HLA-DR positive metastases, 36 shows intermediate levels of antigens, 18 cases expressed high levels, and the rest expressed low levels. HLA-DQ antigens were detectable in only 5 of 13 primary and 8 of 36 metastases tested. In the positive metastatic tumors, 7 of 8 cases were HLA-DQ positive of intermediate level. As for the HMW-MAA the same number of primary tumors expressed high or intermediate levels of positive cells, whereas the highest number of metastatic lesions (12 cases) was found in the high group.

These results indicate that HLA class I antigens were highly expressed on both primary and metastatic melanomas, whereas HLA-DR antigens were observed in the majority of melanomas, particularly the metastatic ones, with intermediate frequency of fluorescent cells. These data also confirm that HLA-DQ antigens are poorly expressed both in primary and metastatic lesions. Overall it appears that the 4 sets of antigens are distributed in a similar fashion among primary and metastatic melanomas.

Phenotypic Heterogeneity of Different Melanoma Metastases Obtained from the Same Patient. In 2 cases the antigenic profile of metastases removed from different anatomic sites of the same patient was analyzed. As shown in Table 3 melanoma cells from 2 lymph node metastases removed a few months apart from the same patient (Me 3338) showed a similar percentage of positive cells for all the antigens tested. A small increase in the percentage of HLA-DR positive tumor cells isolated from lymph node-1 of Me 3338 was seen after in vitro culture (LN-1, C). Conversely in Me 9557 a significant difference in the expression of HLA class II antigens and GD3-MAA was observed between tumor cells from a lymph node metastasis and those isolated from an s.c. metastatic deposit. In this case (Me 9557) a slight increase in the expression of all the antigens tested was seen on cultured cells compared to freshly isolated cells from the same lymph node.

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Modulation of HLA Classes I and II Antigens and MAA by Treatment with IFNs. To determine whether the phenotypic heterogeneity of melanoma cells could be a consequence of the exposure in vivo to soluble products of activated T-cells such as IFN, 12 short-term cultures of metastatic melanoma were treated with human IFN-γ and/or IFN-α.

Doses of IFN-γ or rHuIFN-γ were used in a range between 50–1000 units/ml. A representative dose-response curve is shown in Chart 2. Cells from Me 6155 were exposed for 72 h to different doses of rHuIFN-γ and both HLA classes I and II antigens were then evaluated. Since the majority of tumor cells (83%) were already positive for HLA class I antigens before the treatment, the effect of rHuIFN-γ on the expression of these molecules can be visualized only as a relative increase in fluorescence intensity of the cells (from 142 to >255 with 50 units/ml).

On the contrary, dose-related augmentation in the percentage of HLA-DR positive cells was observed up to 250 units/ml and then remained unchanged. The expression of HLA-DQ antigens reached a maximum at 50 units/ml and did not increase further even when the amount of rHuIFN-γ was augmented up to 1000 units/ml. This was the first indication that in the same tumor rHuIFN-γ could modulate both HLA-DR and -DQ antigens although to a different level.

Table 4 summarizes the results obtained by treating 12 different melanomas with IFNs and then staining them with MoAb to HLA classes I and II antigens, and to GD3 and HMW-MAA. Treatment with IFN-γ induced an increase in the percentage and intensity of cells stained by anti-HLA class I MoAb in all the lesions tested. In particular, in Me 3425 the number of HLA class I positive cells rose from 28 to 60% after IFN-γ treatment. It is noteworthy that even after treatment with IFN-γ the percentage of cells stained by anti-HLA class I MoAb did not reach 100%. rIFN-α was tested with cells from 3 lesions and results were similar to those obtained with rHuIFN-γ. The expression of GD3-MAA and HMW-MAA was not affected by IFN-γ or rHuIFN-γ treatment in four cases tested. In one melanoma (Me 3425), however, both IFN-γ and rIFN-α induced a 2-fold increase in GD3-MAA expression.

A different pattern of results was seen when HLA-DR and -DQ antigens were considered (Table 4). A significant increase in the expression of HLA-DR antigens was seen after either IFN-γ or rHuIFN-γ treatment in all the 12 cases tested.

In one case (Me 9229) HLA-DR expression was increased up to 9-fold with rHuIFN-γ but not with rIFN-α. In 4 other melanomas (Me 28, Me 4322, Me 6155, and Me 3425) a 3-fold increase in HLA-DR expression was observed. An augmentation in fluorescence intensity was always noted even when the percentage of HLA-DR positive cells was already high before the treatment (Me 28 and Me 4322).

Table 4

<table>
<thead>
<tr>
<th>Cells</th>
<th>Treatment</th>
<th>Class I</th>
<th>DR</th>
<th>DQ</th>
<th>MAA</th>
<th>HMW-MAA</th>
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<tbody>
<tr>
<td>Me 9229</td>
<td>Medium</td>
<td>97</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>50</td>
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<tr>
<td></td>
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<td>100</td>
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<td>6</td>
<td>7</td>
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<td>7</td>
<td>2</td>
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<td>26</td>
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<td>IFN-γ</td>
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<td>84</td>
<td>9</td>
<td></td>
<td></td>
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<td>96</td>
<td>207</td>
<td>28</td>
<td>138</td>
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<td></td>
<td>IFN-γ</td>
<td>99</td>
<td>&gt;255</td>
<td>71</td>
<td>220</td>
<td>7  77</td>
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<td>70</td>
<td>37</td>
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<tr>
<td></td>
<td>IFN-γ</td>
<td>95</td>
<td>80</td>
<td></td>
<td></td>
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<tr>
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<td>90</td>
<td>54</td>
<td>23</td>
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<tr>
<td></td>
<td>IFN-γ</td>
<td>95</td>
<td>74</td>
<td>51</td>
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<tr>
<td>Me 6155</td>
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<td>86</td>
<td>142</td>
<td>24</td>
<td>118</td>
<td>7  61</td>
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<tr>
<td></td>
<td>rHuIFN-γ</td>
<td>89</td>
<td>&gt;255</td>
<td>80</td>
<td>250</td>
<td>38  90</td>
</tr>
<tr>
<td></td>
<td>rIFN-α</td>
<td>91</td>
<td>&gt;255</td>
<td>38</td>
<td>103</td>
<td>7  70</td>
</tr>
<tr>
<td>Me 6556</td>
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<td>90</td>
<td>54</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>IFN-γ</td>
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<td>74</td>
<td>51</td>
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<td>Me 3425</td>
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<td>28</td>
<td>94</td>
<td>27</td>
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<tr>
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<td>162</td>
<td>70</td>
<td>108</td>
<td>36  104</td>
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<tr>
<td></td>
<td>rIFN-α</td>
<td>63</td>
<td>157</td>
<td>50</td>
<td>83</td>
<td>16  65</td>
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<td>Normal fibroblasts</td>
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<td>Adherent Mφ (case 1)</td>
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<td>90</td>
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<td>83</td>
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<td></td>
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<td>93</td>
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<td>73</td>
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<td>rIFN-α</td>
<td>95</td>
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</table>

*Cells were treated with rHuIFN-γ or with partially purified IFN-γ at 50–250 units/ml for 72–96 h. rIFN-α was used at 100 units/ml.

Relative fluorescence intensity.
creased by IFN-γ or rhIFN-γ treatment with an increase in HLA-DR antigen expression. Only 1 of 3 melanomas tested respond to IFN-γ or rHulFN-7 treatment with 3429 and Me 906) (data not shown). Thus it appears that all the melanomas tested respond to IFN-γ or rhIFN-γ treatment with an increase in HLA-DR antigen expression. Only 1 of 3 melanomas tested respond to IFN-γ or rHulFN-7 treatment with a significant increase in the percentage of cells stained by anti-HLA-DR MoAb (Me 3425). On the contrary, the expression of HLA-DQ antigens was increased by IFN-γ or rhIFN-γ in 5 of 8 cases (62%); in 2 the increase was about 5-fold and about 3-fold in the remaining 3. Of particular interest is that 3 melanomas (Me 9229, Me 28, and Me 4322) did not show any change in the percentage of cells stained by anti-HLA-DQ MoAb although the percentage of cells stained by anti-HLA-DR MoAb increased between 3- and 9-fold.

Chart 3 represents the FACS histogram of one of these melanomas (Me 9229) treated with IFN-γ. A significant difference in the percentage of HLA-DR positive cells from 10 to 90% and in fluorescence intensity between untreated and treated melanoma cells was observed. On the contrary, HLA-DQ antigen expression remained unchanged. Even by using increasing doses of IFN-γ or rhIFN-γ we were unable to show any augmentation in HLA-DQ antigen expression on cells isolated from Me 9229. These data suggest that different HLA class II antigens on tumor cells can be independently modulated by IFN-γ. Such a conclusion does not apply to normal cells since normal human fibroblasts, adherent monocyte-macrophages, or normal erythrocyte forming rosette-negative lymphocytes respond to the in vitro treatment with IFNs by a parallel increase in the percentage of HLA-DR and -DQ positive cells (Table 4, bottom lines).

Effects of IFN-γ on Growth Kinetics and Antigenic Changes of Melanoma Cells. To exclude that the differences in changes of HLA-DR and -DQ antigen expression did not reflect a shift in the kinetics of melanoma cell growth following IFN-γ treatment, we monitored the DNA content by chromomycin labeling of treated and untreated melanoma cells. Chart 4 shows DNA histograms of Me 9229 cultured in medium or in the presence of IFN-γ (50 units/ml) for 72 h. No change in the cell cycle distribution was seen between treated and untreated cells. The fraction of S-G2-M cells was similar in both groups (33 versus 32%), although a considerable increase in the percentage of HLA class I- and HLA-DR-positive cells occurred at the same conditions (see Table 4 and Chart 3). Therefore we can conclude that the antigenic changes observed by treating with either IFN-γ or rhIFN-γ do not reflect a shift in the growth kinetics of melanoma cells. Moreover these results suggest that HLA-DR and -DQ antigens are not expressed preferentially in a given phase of the cell cycle.

**DISCUSSION**

In the present investigation we have characterized the expression of HLA classes I and II antigens, GD3 MAA, and HMW-MAA on melanoma cells freshly isolated from 20 primary and 73 metastatic lesions. The percentage of positive cases for each of the antigens tested did not differ significantly between primary and metastatic melanomas. However, heterogeneity in the levels of antigen expression was observed within a single tumor and among different melanomas, including different autologous metastases. Similar findings have been reported by others by examining tissue sections and long-term cultured melanoma cell lines (1-11). Specifically HLA class I antigens were present in a high percentage of melanomas derived both from primary (91%) and metastatic (93-98%) lesions. Moreover the tumor cell suspensions versus tissue sections might in part account for these conflicting results. In keeping with our data, however, Natali et al. (1, 8) showed that HLA class I antigens were similarly expressed on tissue sections of primary melanomas and autologous metastases. However, it has to be noted that in every lesion tested HLA class I antigens were not detected on all the melanoma cells. It is not known whether these cells express HLA class I antigens at a level below the sensitivity of our assay or lack them. If the latter is the case, it will be of interest to determine the effect on the interaction of these cells with cytotoxic T-lymphocytes. This appears to be particularly important on the basis of recent findings from our group sug-

**Chart 3.** Effect of IFN-γ on the expression of HLA-DR and -DQ antigens on Me 9229. Cells were incubated for 72 h with medium (top) or with IFN-γ, 50 units/ml (bottom) and then tested using D1-12 (anti-HLA-DR) and TU-22 (anti-HLA-DQ) MoAb and fluorescein isothiocyanate-conjugated F(ab')2 sheep anti-mouse immunoglobulin antibodies. The results are shown as FACS histograms plotting fluorescence intensity versus cell number.

**Chart 4.** DNA histograms from the Me 9229 melanoma cultured in medium (left) or with IFN-γ (50 units/ml) for 72 h (right). Chromomycin labeling was used. N, G1; 2N, G2. S-G2-M, 33% (left) and 32% (right).
sugesting a role for class I antigens in the interaction of cloned cytotoxic T-lymphocytes with autologous melanoma cells (31).

The majority of the tumors examined in the present work expressed variable levels of MAA recognized by MoAb to GD3 (29) and to HMW-MAA (28), thus confirming previous findings of other groups on cryostatic sections or long-term cell lines of human melanoma (1, 29). No significant differences were seen between primary and metastatic lesions. Short-term cultured cells did not show appreciable variation in MAA expression compared to freshly isolated counterparts. However, heterogeneity was evident in GD3 expression when different metastases from the same patient were analyzed.

As for HLA class II antigens, to the best of our knowledge this is the first study in which the simultaneous expression of the products of 2 HLA-D loci, HLA-DR and -DQ, on melanoma cells isolated from primary and metastatic lesions has been analyzed. HLA-DR antigens were expressed in 60% of primary and 50% of metastatic melanomas, whereas HLA-DQ antigens were found in only 38 and 21% of the cases, respectively. The percentage of positive cells varied from tumor to tumor, but the majority of the cases fell in the group with intermediate levels of fluorescent cells (see Chart 1). The relative fluorescence intensities especially for HLA-DQ antigens were usually quite low. Moreover the expression of HLA-DQ was always associated with that of HLA-DR. In no instances was a HLA-DQ positive and HLA-DR negative tumor found, whereas 40–50% of HLA-DR positive melanomas were HLA-DQ negative. Similar observations were recently made for melanoma lines (4, 18) as well as for peripheral blood monocytes (17, 32) and leukemia cells (33). An increased expression of HLA-DR and -DQ antigens was observed in cultured as compared to freshly isolated melanoma cells from which the cultures were derived. These findings may be related to the culture conditions which are known to influence the expression of several surface structures (34, 35). However, an active selection of HLA-DR positive, HLA-DQ positive cells by growth/differentiation factors present in the culture medium cannot be excluded at the present time. Among the soluble factors that are known to influence the expression of surface antigens, IFNs have been studied extensively (15–22). We have analyzed the response to IFNs of 12 short-term cultures of melanoma cells isolated from lymph node metastases. It was found that both rIFN-α and either IFN-γ or rHuIFN-γ treatment induced an increased expression of HLA class I antigens on all melanomas tested. The fluorescence intensity per cell was augmented in all experiments. To the contrary, the effect of either IFN-γ or rHuIFN-γ on HLA class II antigen expression varied depending on the antigen considered. HLA-DR antigens were induced and/or augmented in all melanomas tested. Doses of rHuIFN-γ as low as 20 units/ml were effective in increasing HLA-DR antigen expression after 72 h treatment. The response to IFN-γ was not the result of expanding a DR positive subpopulation because the number of cells recovered from treated cultures was similar or slightly less than untreated ones. The time lag changes between IFN-γ treatment and the phenotyping was too short to allow for the expansion of a selected subpopulation, and HLA-DR antigens were always expressed in 60–90% of IFN-γ treated cells.

However, the increase in HLA-DR antigens was not always associated with an augmentation of HLA-DQ antigen expression. In 3 of 8 cases, the percentage of HLA-DQ positive cells remained unchanged after treatment with IFN-γ or rHuIFN-γ. It should be noted that 3 melanomas that were weakly HLA-DQ positive before treatment showed a 2-fold increase of HLA-DQ expression. Moreover the induction by IFN-γ of HLA-DQ antigens on HLA-DQ negative tumors was observed in 3 cases.

Furthermore the different inducibility of HLA-DR versus HLA-DQ antigens was not due to a shift in the growth kinetics of melanoma cells since by the analysis of the DNA content we did not observe any change in the percentage of S-G2-M between IFN-γ treated and untreated melanoma cells. In case the antiproliferative effect of IFN-γ was negligible due to the short incubation time (72 h) and/or to the low amount of IFN-γ used (50 units/ml). As expected, treatment of normal cells with IFN-γ or rHuIFN-γ resulted in an augmented expression of both HLA classes II and I antigens (15–17) including HLA-DQ products.

All of these data are in agreement with several reports on the effects of different preparations of IFN-γ on the antigenic profile of tumor cell lines of melanocytic origin and of different histology (16, 19–21). There is a general consensus that the effect of IFN-γ on HLA-DQ antigens is more restricted than that on HLA-DR antigens, although the extent of the increase appears to be higher for HLA-DQ than for HLA-DR. All together, these results indicate that the expression of these molecules is under separate genetic control.

The effect of rIFN-α on the expression of HLA class II antigens paralleled those of IFN-γ and rHuIFN-γ only in one of 3 cases examined. In that case, a 2- to 3-fold increase in HLA-DR and -DQ antigens was observed. These results may account for the conflicting data reported in the literature. Several investigators (16, 19–21) failed to see any effect of rIFN-α on the expression of HLA class II antigens on melanoma cell lines, whereas Giacomini et al. (18) showed that high doses of rIFN-α enhanced HLA-DR and -DQ expression on Colo 38, a different melanoma cell line.

It is noteworthy that even following incubation with IFNs, HLA antigens were not detected in 100% of the cells. A certain degree of heterogeneity persisted within each tumor. This might reflect abnormalities in the synthesis, processing, and/or insertion of the antigens on the cell membrane or lack of receptors for IFNs. Moreover, it is not known whether the lack of detectable HLA class II antigens on certain melanomas correlates with the absence of HLA-DR and -DQ mRNA. Since IFNs are both amplifiers of gene expression and inducers of de novo gene transcription (15), further studies on the presence of HLA class II gene transcripts before and after IFN treatment are needed to clarify the molecular mechanism of IFN action on tumor cells.

At the present time, it cannot be excluded that IFN is one of the factors which contribute to tumor heterogeneity in vivo. If this is the case, both positive and negative effects on the host tumor interactions can be envisaged. On the one hand, the enhancement of expression of major histocompatibility complex antigens by IFNs may help to trigger an immunological response to the tumor, since MAA in the context of appropriated major histocompatibility complex antigens would be presented more easily to the immune system. There is evidence in fact that HLA class II positive primary melanomas are better stimulators of autologous lymphocytes responses than are HLA class II negative tumors (12, 14). Metastatic cells, however, either HLA-DR positive or negative are not able to induce activation of autologous lymphocytes, possibly due to the loss of stimulatory MAA antigens (14, 36). On the other hand, it has been shown recently that the increased expression of HLA class II antigens on melanoma cells correlated with inhibition of autologous antitumor
immune response (13) and with augmented metastases (11). Therefore it appears that IFN with its ability to modulate the antigenic profile of tumor cells could favor either positive or negative immune responses in tumor bearing individuals depending on the stage of the disease at which IFN is produced in situ or given as therapeutic agent. Altogether the above findings suggest that caution should be exercised in using IFN in the therapy of malignant diseases.

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Donatella Taramelli, Giuseppe Fossati, Arabella Mazzocchi, et al.


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