Differential Modulation by Recombinant Immune Interferon of the Expression and Shedding of HLA Antigens and Melanoma Associated Antigens by a Melanoma Cell Line Resistant to the Antiproliferative Activity of Immune Interferon

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ABSTRACT

By culture of human melanoma Colo 38 cells in the presence of increasing concentrations of recombinant immune interferon (IFN-γ), the clone RZ-γ-4G1 resistant to the antiproliferative action of IFN-γ (3 × 10^4 units/ml) was isolated. This clone was cultured for 6 weeks in the absence of IFN-γ and was subsequently treated with increasing concentrations of IFN-γ. Contrary to the response of its parental cell line, treatment of this clone with IFN-γ did not significantly alter the rate of protein or DNA synthesis and did not markedly modulate the cell surface expression of HLA Class I antigens, of the high molecular weight melanoma associated antigen, and of a M, 100,000 melanoma cell line resistant to the antiproliferative action of IFN-γ. IFN-γ caused an increase in the cell surface expression and in the shedding of HLA Class II antigens from IFN-γ resistant cells. Four proteins with molecular weights of 32,000, 38,000, 46,000, and 50,000 were induced by IFN-γ in the parental melanoma cells but not in the resistant clone. Both cell lines bound equivalent amounts of ^125I-IFN-γ to their surface, indicating that the lack of specific surface receptors was not the cause of insensitivity to IFN-γ. These results indicate that at the cellular level IFN-γ modulates the expression and shedding of HLA Class II antigens through different mechanisms to those responsible for the antiproliferative action, modulation of the cell surface expression of melanoma associated antigen and of HLA Class I antigens, and induction of new proteins in cultured melanoma cells. Since the success of therapy of malignant diseases with IFN-γ depends on the extent of resistance of individual tumor cells, the present study may provide a better understanding of the biology of IFN-γ insensitive tumor cells and particularly the malignant melanoma.

INTRODUCTION

Because of the potential application in the therapy of malignant diseases, the effects of immune interferon (IFN-γ) on tumor cells have been characterized. It has been reported that IFN-γ inhibits the proliferation of cultured tumor cells (1, 2), enhances the cell surface expression of HLA Class I and Class II antigens on melanoma cells (3–6), reduces the expression of the HMW-MAA (2), and induces new cellular proteins in tumor cells (7). The mechanisms by which IFN-γ induces various biological responses in the target tumor cells are not understood, and it is not yet clear whether various actions of IFN-γ on target tumor cells are mediated via independent processes or whether such responses are linked phenomena. To answer these questions two separate approaches can be used. At the molecular level, monoclonal antibodies directed against distinct determinants of the IFN-γ molecule may be used to study the responses of the target tumor cell to IFN-γ treatment. Using such an approach, Rubin et al. (7) showed that the antiproliferative and protein inducing actions of IFN-γ are mediated through the same functional domains of the IFN-γ molecule. Celada et al. (8) showed in the murine system that the antiviral and macrophage activation properties of IFN-γ were separate processes. We have shown that the functional domains responsible for the antiproliferative, enhanced expression of HLA Class I and Class II antigens and antiviral activities of recombinant human IFN-γ are distinct from those responsible for the reduction in the expression of the HMW-MAA and the binding of IFN-γ to cell surface receptors.

A second approach is to isolate variants of tumor cell lines which show insensitivity to one or more actions of IFN-γ. The most practical selection system is the resistance to the antiproliferative action of IFN-γ. Such an approach was used (9–11) to isolate clones of human tumor cell lines resistant to leukocyte IFN (IFN-α). One clone of the Burkitt lymphoma cell line Daudi which was resistant to the antiproliferative action of IFN-α and fibroblast interferon (IFN-β) was isolated by Dron and Tovey (10) and was shown to be also resistant to the antiviral actions of IFN-β. This cell line bound equal amounts of IFN-α to its receptors and was shown to have high affinity receptors similar to those present on the parental Daudi cells (12).

In this study, we have used the anticalcellular selection system to isolate a clone of human melanoma cell line which was resistant to the antiproliferative action of recombinant IFN-γ (3 × 10^4 units/ml). We show that the isolated resistant clone can specifically bind an equivalent amount of IFN-γ as its parental melanoma cell line and that the insensitivity to the antiproliferative action of IFN-γ is associated with resistance to the influence of IFN-γ on the cellular protein and DNA synthesis, induction of new proteins, modulation in the expression of the HMW-MAA and of the M, 100,000 MAA as well as in the expression and shedding of HLA Class I antigens. The mechanism(s) underlying
these effects of IFN-γ appear to be different from those mediating the enhanced expression and shedding of HLA Class II antigens.

**MATERIALS AND METHODS**

**Cell Line.** Cultured human melanoma Colo 38 cells were grown at 37°C in DMEM supplemented with 10% fetal calf serum, 2.0 mM L-glutamine, and gentamicin sulfate (10 µg/ml).

**Interferons.** Human recombinant IFN-α, a generous gift from Dr. S. Pestka, Roche Institute of Molecular Biology, Nutley, NJ, was produced in Escherichia coli and was purified to homogeneity as described (13). The IFN-α preparations were aliquoted, stored at −80°C, thawed immediately before use, and diluted to the desired concentration in DMEM. Human recombinant IFN-γ, a generous gift from Dr. C. G. Sevastospolous, Genentech, Inc., San Francisco, CA, was produced in Escherichia coli and purified to homogeneity (14). It had an initial antiviral activity of 6.8 × 10^4 units/ml. Dilutions were prepared in complete DMEM at 1 × 10^6 units/ml prior to use. IFN-γ was labeled with 125I by the iodogen method (15) to a specific radioactivity of 6000 cpm/µg of protein.

**Monoclonal Antibodies and Conventional Antibodies.** The anti-HMW-MAA MoAb 225.285, the anti-HLA Class I MoAb CR11-463, the anti-β2-microglobulin MoAb NAMB-1, and the anti-HLA Class II MoAbs Q2/80 and Q5/10 were prepared and characterized utilizing a methodology described elsewhere (16-18). The MoAb CL203 is secreted by a hybridoma constructed with splenocytes from a BALB/c mouse immunized with IFN-γ treated melanoma cells (Colo 38). The antibody recognizes a Mr. 100,000 MAA with differential expression on primary and metastatic melanoma lesions. Monoclonal antibodies were purified by caprylic acid precipitation (19) and radioiodinated by the iodogen method. Affinity purified rabbit anti-mouse IgG antibodies (Cappel Laboratories, West Chester, PA) were radioiodinated by the iodogen technique to a specific radioactivity of 9000 cpm/µg.

**Measurement of the Incorporation of [3H]Thymidine and [3C]-Amino Acids.** The incorporation of [3H]thymidine into total cellular DNA was measured as described by Jasny et al. (20). The incorporation of [3C]-amino acids (New England Nuclear, Boston, MA) was measured by treating cultured cells (2 × 10^6 cells/ml) with IFN-γ for 24 h and the labeling of cultured cells with [3C]-amino acids to a final concentration of 2 µCi/ml at 30-min intervals for 3 h. Cells were solubilized in 1% SDS, and the lysate was precipitated with 10% TCA, dissolved in SDS at 100°C, and counted in a β scintillation counter.

**Direct Binding Assay of IFN-γ.** Cells were resuspended in PBS-1% bovine serum albumin-0.2% sodium azide and placed in a microtiter plate (2 × 10^4 cells/50 µl/well). Target cells were incubated with 18,000 cpm of 125I-IFN-γ in 50 µl of PBS for 24 h at 37°C, the assay plate was centrifuged, cells were washed six times with PBS-bovine serum albumin buffer and dried, and the cell bound radioactivity was measured in a gamma-counter. The nonspecifically bound radioactivity was measured by preincubating target cells with 100-fold excess of IFN-γ prior to the addition of 125I-IFN-γ and counting the cell bound radioactivity.

**Serological Assays.** All assays were carried out in flexible microtiter plates. The indirect binding assay was carried out by sequential incubation of target melanoma cells with monoclonal antibodies and 125I-rabbit anti-mouse IgG antibodies as described by Williams (21). The double determinant immunoassay used to measure HLA antigens in culture supernatants was performed as described (22, 23) except for the incubation of culture supernatants with the solid phase antibody for 48 h at 4°C.

**Intrinsic Labeling of Melanoma Cells with [35S]Methionine and 2D-PAGE Analysis.** Melanoma cells were cultured for 24 h in methionine-free RPMI 1640 (Flow Laboratories, McLean, VA) supplemented with 1% fetal calf serum and IFN-γ (1000 units/ml). Cultured cells were labeled for 4 h with [35S]methionine (5 µCi/ml) and solubilized in 1% SDS for 30 min at 4°C. Cell debris was removed by centrifugation and supernatants were precipitated with cold 10% TCA. Precipitates were washed in cold acetone, redissolved in 1% SDS and 1% 2-mercaptoethanol, and heated to 100°C for 5 min. The 2D-PAGE analysis of cell extracts was performed as described by O’Farrell (24) with minor modifications (25). Nonequilibrium 2D-PAGE was performed as described by O’Farrell et al. (26). Gels were autoradiographed as described by Laskey and Mills (27).

**RESULTS**

Colo 38 melanoma cells were cloned by limiting dilution (28) in four microtiter plates and cultured in the presence of IFN-γ (final concentration, 5000 units/ml). Wells of culture plates were examined regularly for active growth and confluent wells were expanded to 1-ml and subsequently to 10-ml cultures in the presence of IFN-γ (5000 units/ml). No feeder layers or conditioned media were used for the cloning and expansion. Cells were subcloned when cultures reached a density of 1 × 10^6 cells/ml. This process was repeated three more times in the presence of IFN-γ (1 × 10^6, 2 × 10^6, and 3 × 10^6 units/ml). Following the final subcloning only two culture wells showed active proliferation in the presence of IFN-γ (3 × 10^4 units/ml). One of them, coded RZγ4G.1, was expanded in culture and grown in complete DMEM without added IFN-γ for 6 weeks before being characterized. The anti-proliferative action of IFN-γ on the resistant RZγ4G.1 and its parental cell line Colo 38 was investigated by culturing the cells for 72 h in the presence of increasing concentrations of IFN-γ (0–2000 units/ml). As shown in Chart 1, the parental cell line Colo 38 showed a dose-dependent decrease in proliferation; at a 1000-unit/ml dose of IFN-γ, the cellular proliferation was completely inhibited. The resistant RZγ4G.1 cells were not significantly affected by IFN-γ up to a concentration of 2000 units/ml. The viability of both cell lines in all cultures was over 90% before and after IFN-γ treatment. The RZγ4G.1 cells generally grew less rapidly and were less adherent than the parental cells (Colo 38). Furthermore contrary to the behavior of Colo 38 cells, the RZγ4G.1 cells did not grow in roller cultures.

The effect of IFN-γ on the protein and DNA synthesis by cultured melanoma cells Colo 38 and RZγ4G.1 was examined by growing the cells for 24 h in the presence of IFN-γ (1000 units/ml) followed by labeling with either [3H]thymidine or [3C]-amino acids.
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amino acid mixture. Following solubilization of the labeled cells and precipitation of the incorporated radioactivity with TCA, IFN-γ treatment of Colo 38 cells caused a 30% reduction in total cellular protein synthesis and a 17% decrease in the total DNA synthesis (Table 1). On the other hand, the same concentration of IFN-γ caused only a 7% reduction in cellular protein synthesis and a 4% decrease in the incorporation of [3H]thymidine into DNA by RZγ-4G.1 cells. IFN-γ at concentrations above 1000 units/ml always caused a reduction in the total cellular protein synthesis whereas a significant inhibition of DNA synthesis was observed only when cultured melanoma cells were in the log phase of growth prior to IFN-γ treatment (results not shown).

In order to examine whether the cause of insensitivity of the RZγ-4G.1 cells to IFN-γ was the lack of specific binding sites, binding of 125I-labeled IFN-γ to these cells and the parental cell line Colo 38 was measured. The cultured cells were first treated with 0.2% sodium azide to inhibit the internalization of the IFN-receptor complex (29) and then incubated with 125I-labeled IFN-γ. As shown in Chart 2, both cell lines bound equivalent amounts of IFN-γ to their surface. The level of binding did not increase when the amount of added 125I-labeled IFN-γ was increased by 2-fold, suggesting that the cell surface receptors were saturated by the amount of IFN-γ added. The nonspecifically bound radioactivity, however, became proportionally higher with the increased amount of added 125I-IFN-γ (not shown).

The effect of IFN-γ on the expression of HLA Class I and Class II antigens and of HMW-MAA on the RZγ-4G.1 and Colo 38 cells was investigated by testing cells cultured for 48 h in the presence of increasing concentrations of IFN-γ (0–2000 units/ml). In order to eliminate the differences in the baseline radioactivity for each cell line, the results are expressed in terms of ratios of the cell bound radioactivity at each IFN-γ dose to that bound to the control cells. Treatment of Colo 38 cells with IFN-γ caused a marked increase in the expression of HLA Class I and Class II antigens in a partially dose dependent manner reaching an IFN-γ maximum at 1000 units/ml. The IFN-γ resistant RZγ-4G.1 cells treated with the same concentrations of IFN-γ showed an enhanced expression of HLA Class II antigens but only a weak increase in the expression of HLA Class I antigens (Chart 3). Treatment of Colo 38 cells with IFN-γ caused a sharp increase in the expression of the M, 100,000 MAA recognized by MoAb CL203 and a decrease in the expression of the HMW-MAA recognized by MoAb 225.28S (Chart 4). These changes were also partially dose dependent reaching a maximal IFN-γ effect at 1000 units/ml. The RZγ-4G.1 cells treated with the same concentrations of IFN-γ showed no significant increase in the expression of the MAA detected by MoAb CL203 and only a slight decrease in the expression of the HMW-MAA (Chart 4).

Table 1
Effect of IFN-γ on the rate of protein and DNA synthesis by cultured melanoma Colo 38 and RZγ-4G.1 cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IFN-γ (units/ml)</th>
<th>Total TCA precipitable radioactivity/1 × 10⁶ cells</th>
<th>[14C]-amino acids (10⁶ x cpm)</th>
<th>[3H]-Thymidine (10⁶ x cpm)</th>
</tr>
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<tbody>
<tr>
<td>Colo 38</td>
<td>0</td>
<td>452.1 ± 14.8a</td>
<td>25.1 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Colo 38</td>
<td>1000</td>
<td>312.3 ± 6.2</td>
<td>20.8 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>RZγ-4G.1</td>
<td>0</td>
<td>252 ± 5.3</td>
<td>27.9 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>RZγ-4G.1</td>
<td>1000</td>
<td>235 ± 7.1</td>
<td>26.8 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD of three replicate wells in each determination.

The specific cell bound radioactivity for Colo 38 (○) and RZγ-4G.1 (△) cells are presented as means of eight replicate assay wells; bars, SD.
In order to investigate the effect of IFN-γ on the shedding of HLA antigens, the melanoma cells Colo 38 and RZγ-4G.1 were cultured for 48 h in the presence of increasing concentrations of IFN-γ (0–2000 units/ml). The culture supernatants were collected and tested utilizing a double determinant immunoassay. The results have been adjusted to account for the differences in the proliferation of Colo 38 and RZγ-4G.1 cells in the presence of IFN-γ and are summarized in Chart 5. The shedding of HLA Class I and Class II antigens from melanoma cells Colo 38 was increased by IFN-γ in a dose dependent fashion. The resistant RZγ-4G.1 cells treated with the same concentration of IFN-γ showed an increase in the shedding of HLA Class II antigens but not of HLA Class I antigens. The viability of all cell cultures was greater than 90% before and after IFN-γ treatment as determined by trypan blue exclusion test. Since all classes of IFNs have been reported to induce new intracellular proteins in murine and human tumor cells (30–33), we examined this particular action of IFN-γ on the melanoma cells Colo 38 and RZγ-4G.1. Cells were cultured for 24 h in methionine free medium RPMI 1640 containing IFN-γ (1000 units/ml). Cells were subsequently labeled with [35S]methionine, total cell extracts were run on 2D-PAGE, and gels were autoradiographed. As shown in Fig. 1b, IFN-γ treatment of Colo 38 cells induced four proteins with neutral isoelectric points and molecular weights of 32,000, 38,000, 46,000, and 50,000. These proteins were undetectable in the two-dimensional gel of the extract from the control Colo 38 cells (Fig. 1a) and the extract from the resistant RZγ-4G.1 cells treated with IFN-γ (1000 units/ml) (Fig. 1c). These induced proteins are likely to be intracellular species since they were undetectable in the solubilized membrane preparations run on 2D-PAGE. They were also undetectable in Colo 38 cells treated with IFN-α (1000 units/ml) (results not shown). In order to ensure maximum detectability of the faint spots, the total amount of TCA precipitable radioactivity loaded on the two-dimensional gels of RZγ-4G.1 cell extract was twice that loaded on the other gels shown in Fig. 1. Furthermore the labeled extract of the RZγ-4G.1 cells was run on the nonequilibrium 2D-PAGE but none of the four proteins shown in Fig. 1b was detectable in the extract of IFN-γ treated RZγ-4G.1 cells (data not shown). These proteins were not induced in Colo 38 cells by IFN-γ preparations which had lost their ability to inhibit cellular proliferation or modulate the expression of cell surface antigens in Colo 38 cells (results not shown).

Throughout the present study, there were no significant indications of reversion of RZγ-4G.1 cells to IFN-γ sensitivity. To ensure that the degree of resistance remained high, however, the RZγ-4G.1 cells were periodically treated with IFN-γ (1 x 10^4 units/ml) for 5 days and subsequently passaged in regular culture medium. The cultured cells were tested for resistance to the

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**Chart 5. Effect of IFN-γ on the shedding of HLA Class I and Class II antigens by melanoma cells Colo 38 and RZγ-4G.1.** Cultures of melanoma cells Colo 38 (—) and RZγ-4G.1 (-----) were added with increasing amounts of IFN-γ. Following a 48-h incubation at 37°C, spent medium was harvested and tested in triplicate for its content of HLA Class I antigens (○) and of HLA Class II antigens (□). The double determinant immunoassays for HLA Class I and Class II antigens were performed utilizing the combinations of insolubilized MoAb CR11-463-125-MoAb NAMB-1 and insolubilized MoAb Q5/13-125l-MoAb 02/80, respectively. The data presented are averages of three replicate determinants with a less than 5% variation among replicates. U, units.

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**Fig. 1.** 2D-PAGE of [35S]methionine labeled extracts of Colo 38 (a), of Colo 38 treated with IFN-γ (1000 units/ml) for 24 h (b), and of RZγ-4G.1 cells treated with IFN-γ (1000 units/ml) for 24 h (c). Small arrows, position of reference spots used to localize spots induced by IFN-γ treatment of the cells (larger arrow). Ordinate, positions of the protein molecular weight markers (in thousands): 92, phosphorylase b; 66, bovine serum albumin; 45, ovalbumin; 30, carbonic anhydrase; 21, trypsin inhibitor; and 14, lysozyme. The acid end of the pH gradient is on the left of the figures and the SDS-PAGE was performed from the top to the bottom of the two-dimensional gels. IEF, isoelectric focusing.
antiproliferative action of IFN-\(\gamma\) (1000 units/ml) at regular intervals.

DISCUSSION

The object of the present study was to examine a possible relationship between the antiproliferative action of IFN-\(\gamma\), its influence on cellular protein and DNA synthesis, and its effect on the expression and shedding of surface antigens in human melanoma cells in culture. The results presented above indicate that there appears to be a link between the antiproliferative activity of IFN-\(\gamma\) and its inhibitory action on the cellular protein and DNA synthesis, since a clone of human melanoma cell line Colo 38 with IFN-\(\gamma\) resistant phenotype was shown to be unaffected by IFN-\(\gamma\) in these properties. Interferons have been reported to inhibit division of tumor cells by prolonging their intermitotic time and either the \(G_1\) and \(G_2\) phase (34) or the \(G_0\) and the S phase of the cell cycle (35). This may imply that the IFN-\(\gamma\) resistant cells enter the various stages of the cell cycle at normally designated periods and that the block in \(G_0\) or \(G_2\) (or both) induced by IFN-\(\gamma\) on the sensitive cells is not operative in the resistant tumor cells. Since in our study both the parental cell line Colo 38 and the IFN-\(\gamma\) resistant cell line RZ\(\gamma\)-4G.1 were regularly passaged and were in a state of active division and growth before each experiment, it is unlikely that cellular aging played a significant role in the observed reduction in the cellular protein and DNA synthesis, as described for the effect of IFN-\(\beta\) on human fibroblasts at different population doubling levels (20).

An essential step in the biological action of all classes of IFNs is their specific binding to surface receptors on target cells (36, 37) and possibly internalization of the IFN-receptor complex (29). The IFN-\(\alpha\) resistant mouse L1210 cells do not express IFN-\(\alpha\) receptors and this accounts for their unresponsiveness to IFN-\(\alpha\) (38). However, several IFN resistant cells have equal or more capacity for specifically binding IFN to their surface receptors (10, 39, 40). It has been suggested that IFN resistant cells may have lost a high affinity interaction between cellular receptors and IFN (41). Hence the equivalent binding of IFN-\(\gamma\) to the melanoma cells Colo 38 and RZ\(\gamma\)-4G.1 may indicate that both cells have an equal number of IFN-\(\gamma\) receptors. It remains to be determined whether the affinity of such receptors on IFN-\(\gamma\) resistant cells may be low and therefore prevent IFN-\(\gamma\) to be in direct contact with the cell membrane for a sufficient time. We also examined the modulation by IFN-\(\gamma\) of the cell surface expression of MAA and HLA antigens. Our data indicate that the expression of HLA Class II antigens is increased by IFN-\(\gamma\) treatment of melanoma cells Colo 38 and RZ\(\gamma\)-4G.1 whereas a significant increase in the expression of HLA Class I antigens appears to be restricted to IFN-\(\gamma\) sensitive Colo 38 cells.

Our results suggest that different mechanisms are responsible for the increase in the expression of HLA Class I and Class II antigens and that the resistance to the antiproliferative action of IFN-\(\gamma\) may be linked to the insensitivity of melanoma cells to IFN-\(\gamma\) with respect to a modulation in the expression of HLA Class I antigens. The increase in the expression of the M\(_{100,000}\) MAA recognized by MoAb CL203 and the decrease in the expression of HMW-MAA also appear to be related to the degree of IFN-\(\gamma\) resistance by cultured melanoma cells. Our study also shows that IFN-\(\gamma\) increases the shedding of HLA Class I and Class II antigens from viable Colo 38 melanoma cells but has a differential effect on the shedding of HLA Class I and Class II antigens from the resistant cells, RZ\(\gamma\)-4G.1. Only the shedding of HLA Class II antigens is related to the insensitivity of cultured melanoma cells to the antiproliferative activity of IFN-\(\gamma\). The viability of the melanoma cells cultured in the presence of IFN-\(\gamma\) up to a concentration of 2000 units/ml was always greater than 90%; therefore the shedding of HLA antigens was not the result of autolysis of melanoma cells. The failure of the IFN-\(\gamma\) resistant cells to shed HLA Class I antigens may have significance in their in vivo ability to metastasize, since the metastasizing properties of tumor cells appear to be influenced by the extent of shedding of transplantation antigens in the murine system (42, 43). We also examined the induction of new cellular proteins by IFN-\(\gamma\) in the sensitive melanoma cells Colo 38 and IFN-\(\gamma\) resistant RZ\(\gamma\)-4G.1. Four proteins were induced in the Colo 38 cells treated with IFN-\(\gamma\) which were undetectable in the RZ\(\gamma\)-4G.1 cells incubated with the same concentration of IFN-\(\gamma\). All classes of IFNs have been reported to induce proteins in their target tumor cells (30–33). The biological functions of these proteins are unknown, but recently Horisberger and Hochkeppel (44) showed that a protein induced by IFN-\(\alpha\) and IFN-\(\beta\) in mouse cells is involved in the mechanism of resistance to influenza virus. The four proteins induced by IFN-\(\gamma\) in Colo 38 cells are not inducible by IFN-\(\alpha\) and their lack of induction in the IFN-\(\gamma\) resistant RZ\(\gamma\)-4G.1 cells indicates that this property of IFN-\(\gamma\) is also linked to its antiproliferative action.

At present we do not know whether the IFN-\(\gamma\) insensitivity by the melanoma cells RZ\(\gamma\)-4G.1 is the result of genetic mutation or the induction of resistance by IFN-\(\gamma\) itself. The frequency of IFN-\(\gamma\) resistant melanoma cells is far too great to be due to spontaneous mutation (i.e., 0.5%). However, it should be remembered that tumor cells are aneuploid and conventional genetics of normal diploid cells may not be strictly applicable to these cells. Furthermore in all studies where IFN resistant clones were isolated a similar degree of frequency was obtained (10, 11). Besides the possibility of mutation, the induction of resistance by IFN-\(\gamma\) itself must be considered. Whether IFN-\(\gamma\) resistance is a result of genetic mutation or induction is certainly worthy of extensive research, since a successful use of IFN-\(\gamma\) in the treatment of human cancer would largely depend on the risk to select tumor cell populations with a highly malignant phenotype.

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