Induction of Class I Major Histocompatibility Complex Antigens in Human Teratocarcinoma Cells by Interferon without Induction of Differentiation, Growth Inhibition, or Resistance to Viral Infection

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ABSTRACT

The behavior of human teratocarcinoma cells, and especially their stem cells (embryonal carcinoma cells), may provide insights into the properties of human early embryonic cells. We report here that human recombinant γ-interferon (IFN-γ) induced the expression of major histocompatibility complex Class I (HLA-A, B, C) antigens and β2-microglobulin in the two human embryonal carcinoma cell lines, 2102Ep cl.4D3 and NTERA-2 cl.D1, and in the yolk sac carcinoma cell line, 1411H; human recombinant IFN-α and IFN-β were less effective inducers of these cell surface molecules. No induction was observed in the gestational choriocarcinoma cell line, JAR. Neither IFN-α, IFN-β, nor IFN-γ caused growth inhibition, expression of major histocompatibility complex Class II (HLA-DR) antigens, resistance to viral (vesicular stomatitis virus) infection, or expression of 2',5'-oligo(A) synthetase in any of the cells. Also, IFN-γ neither induced differentiation of NTERA-2 cl.D1 cells, which are pluripotent human stem cells, nor influenced their differentiation induced by retinoic acid. However, developmental regulation of responsiveness to IFN was evident, since IFN-γ induced higher levels of surface expression of HLA-A, B, C and β2-microglobulin in the retinoic acid-induced differentiated NTERA-2 cl.D1 cells than in the undifferentiated parental cells. Also, 2',5'-oligo(A) synthetase was inducible in the NTERA-2 cl.D1 differentiated cells by IFN-α and -β, although not by IFN-γ, and slight resistance to vesicular stomatitis virus infection was evident in aged cultures of differentiated cells exposed to IFN-α. The effect of recombinant mouse IFN-γ on major histocompatibility complex expression by several murine teratocarcinoma cells was also examined: H-2 Class I (H-2P), but not class II (I-A), antigens were induced in the parietal yolk sac carcinoma lines, PYS and F9Ac cl.3P; in cultures of PCC4azaR containing both embryonic carcinoma (EC) and differentiated cells; and in cultures of the EC cells, PCC4azaR and PCC4AO, without evidence of differentiation. No induction was observed in the murine EC cell lines, F9 or FA (H-2K). Our results indicate that human EC cells, like murine EC cells, exhibit only a partial response to the interferons, and that the extent of this response is developmentally regulated.

INTRODUCTION

IFNs are cytokines with pleiotropic effects on cellular physiology. First identified by their ability to protect cells from viral infection (1), IFNs have since been shown to inhibit growth of many normal and neoplastic cells, to affect the expression of cell surface antigens, notably by inducing MHC antigens, and, especially in the case of IFN-γ, to play a regulatory role in the hematopoietic system (for reviews, see Refs. 2 to 4). In the latter case, for example, IFN-γ has been shown to induce differentiation of both normal and neoplastic cells of the myeloid lineages (5). IFN-γ has also been reported to be a weak inducer of globin mRNA in some lines of Friend erythroleukemia cells (6) and to inhibit the adipose conversion of 3T3 cells (7). These observations, together with the findings that the ability to synthesize and to respond to IFN is developmentally regulated during mouse embryogenesis (8, 9) and during the differentiation of murine teratocarcinoma cells (10, 11), have suggested that the IFNs may play some role in the regulation of cellular differentiation. Moreover, it has been suggested that alteration of physiological properties of embryonic cells by IFN could be involved in the mechanisms whereby some teratogenic viruses cause abnormalities in embryonic and fetal development (9).

In the mouse, EC cells, the stem cells of teratocarcinomas, exhibit properties akin to those of the primitive ectoderm of the early embryo (12). They have been used extensively as models to investigate aspects of cellular differentiation during embryogenesis (13). Initial studies indicated that murine EC cells do not respond to IFNs by developing an antiviral state, although their differentiated derivatives do respond in this way (10). This observation correlates with the finding that antiproliferative effects of IFN on murine embryos could not be detected until at least 8 days of development (8). Subsequently, it was found that, in murine EC cells, IFNs induce the appearance of 2-5A synthetase, an enzyme associated with the antiviral response in other cell types (11). It was further found that, in at least one EC cell line, PCC4azaR, the appearance of 2-5A synthetase did correlate with IFN-induced resistance to one group of viruses, the picornaviruses, but not other viruses such as VSV (14).

As murine teratocarcinoma cells have provided models for the study of mouse embryogenesis, so human teratocarcinomas provide a necessary model with which to investigate the properties of human embryonic cells (15). A number of studies already indicate the results obtained with one species cannot be directly extrapolated to the other and that human EC cells differ in many ways from their murine counterparts (16–19). Therefore, we have studied the effect of IFNs on several human teratocarcinoma cell lines, including the EC lines, 2102Ep and NTERA-2 (18, 20), and the yolk sac carcinoma line, 1411H (21), and also the gestational choriocarcinoma line, JAR (22). We have also compared the effect of IFN-γ on the expression of MHC antigens in teratocarcinoma cells of both human and mouse origin.

MATERIALS AND METHODS

Cells and Cell Culture. All cells were grown in the high glucose formulation of Dulbecco’s modified Eagle’s medium, supplemented with 10% (v/v) fetal calf serum, at 37°C under a humidified atmosphere containing 5% CO2 in air. For experimental analyses (e.g., for surface antigen expression) cells were harvested (unless otherwise stated) by brief exposure to 0.25% trypsin: 2 mM EDTA in calcium- and magnesium-free Dulbecco’s phosphate-buffered saline (16, 18). The clonally
RESPONSE OF TERATOCARCINOMA CELLS TO IFN

derived human EC lines, 2102Ep cl.4D3 and NTERA-2 cl.D1 (abbreviated NT2/D1), were maintained at a high cell density (>5 x 10^6 cells per 75-cm² flask) by subcultivation twice a week, as previously described (18, 20). NT2/D1 cells were induced to differentiate by seeding 10⁵ cells per 75-cm² flask in medium containing 10⁻³ M retinoic acid (23). The other human cell lines studied were the yolk sac carcinoma line, 14111H (21), the gestational choriocarcinoma line, JAR (22), and the HeLa derivative, HEp2.5 (24). 14111H cells were subcultivated weekly at 1:3 split ratios; JAR and HEp2.5 were subcultivated weekly at 1:10 to 1:20 split ratios. Several murine teratocarcinoma lines were also studied; their characteristics and the relevant references are indicated in the Table 1 legend.

Interferon. Recombinant human IFN-α (subtype α2, or A) was from Hoffmann-LaRoche, Inc., Nutley, NJ; recombinant human and mouse IFN-γ were supplied by Genentech; and recombinant human IFN-β was supplied by Cetus Corporation, Emeryville, CA. Interferon antiviral activity was determined by protection of human fibroblast strain Detroit 532 or murine I.L929 cells from the cytopathic effect of VSV. IFN-α and -β were compared to the NIH IFN-α standard, G-023-901-527, and human IFN-γ was compared to the NIH IFN-γ standard, Gg-23-901-530. Sterile, stock solutions of 10⁴ antiviral units/ml were prepared in medium and stored at −70°C (IFN-α or IFN-β) or at 4°C (IFN-γ); dilutions were prepared from the stock immediately before seeding the cells.

Cell Surface Antigens. Cell surface antigen expression was determined by indirect immunofluorescence assay mediated by flow cytometry on an Ortho Cytotfluorograf 50H, as previously described (16, 18). The following monoclonal antibodies were used: anti-SSEA-1 (25); anti-SSEA-3 (26); TRA-2-54/2J (anti-liver alkaline phosphatase) (27); A2B5 (antiganglioside GQ06) (28); ME311 (antiganglioside 9-O-acetyl GD3) (29); N901 (30); W6/32 (anti-HLA-A, B, C) (31); B.BM1 (anti-β₂-m) (32); DA-2 (anti-HLA-DR) (32); D1-12 (anti-HLA-DR) (33); B22-249R1 (anti-H-2D) (34); 11.4.1 (anti-H-2K) (35); and 11-54.3 (anti-Ia, recognizes a common determinant of I-A and I-E of many II haplotypes including I-A' (36)). For negative controls, the antibody B22-249R1 (anti-H-2D) (34) was used. The fluorescent secondary antibody was fluorescein-conjugated goat anti-mouse IgG or IgM, or goat anti-rat IgG (Capp Laboratories, Cochranville, PA), diluted 1:10.

VSV Infection and Titration. VSV stocks were obtained by growth in a rhabdomyosarcoma-derived cell line: supernatant from infected cultures was recovered and stored at −70°C. The virus content of this stock was assayed by titration on HEp₂.5 cells; the log₁₀ TCID₅₀ was 5.0.

To assay the antiviral effect of IFN, cells were seeded in 96-well microtiter tissue culture plates at 10⁵ cells per well; 1 day later, cells were fed with 0.1 ml of medium containing IFN and then infected with VSV (0.1 ml, 1:1000 dilution of stock virus) on the following day. The virus was allowed to adsorb to the cells for 2 h, after which the cells were washed 5 times before culturing for a further 2 days in 0.2 ml fresh medium; the final wash was saved and titrated to estimate the residual input virus. VSV produced a marked cytopathic effect on all cells tested; after 2 days all the cells in cultures not exposed to IFN were rounded up and, in many cases, detached from the substrate. The extent of the cytopathic effect was estimated by microscopic examination in comparison with uninfected controls. The virus yields of infected wells were asayed by infecting HEp₂.5 cells (seeded as above, but without IFN) with serial 10-fold dilutions of culture supernatants in quadruplicate. After 2 days the presence of a cytopathic effect was ascertained for each dilution of input virus, and the log₁₀ TCID₅₀ was calculated by the method of Reed and Muench (38). A difference of >1 log₁₀ unit between assays was considered significant.

2-5A Synthetase. Cell cultures were grown in the presence or absence of IFN for 24 h, harvested by scraping, washed twice with phosphate-buffered saline, and snap-frozen. Extracts were prepared and assayed for 2-5A synthetase activity as previously described (14).

RESULTS

Effect of IFN-γ on Human EC Cell Growth and Differentiation. We first tested NT2/D1 pluripotent human EC cells to determine whether IFN-γ would either induce the differentiation of these EC cells, or whether it would modify their differentiation induced by retinoic acid (Fig. 1). Retinoic acid alone causes a reduction in cell growth rate, together with changes in morphology and in surface antigen expression; SSEA-3 and an antigen associated with liver alkaline phosphatase are lost or reduced in activity, while SSEA-1, A2B5, and ME-311 are induced (23, 39, 40). Another antigen, not previously studied in this system, defined by monoclonal antibody N901 and also expressed by natural killer cells (30), is also induced upon retinoic acid-induced differentiation of NT2/D1 cells. Cell growth and expression of these marker antigens were not significantly affected by 100 units/ml of IFN-γ, in the absence or presence of 10⁻³ M retinoic acid (Fig. 1). However, IFN-γ did have a marked effect on HLA expression, strongly inducing the monomorphic HLA-A, B, C antigen detected by the monoclonal antibody W6/32 in both the EC cells and their retinoic acid-induced differentiated derivatives. Concentrations of retinoic acid lower than 10⁻⁴ M fail to induce differentiation of all the cells in cultures of NT2/D1 as indicated by the persistence of SSEA-3-positive cells (23); the presence of IFN-γ did not alter the relative proportions of differentiated and EC cells in cultures exposed for 7 days to retinoic acid down to 10⁻¹⁰ M (data not shown). In addition to changes in surface antigen expression, the appearance of neurons characterized by their expression of neurofilament proteins (23, 41) is a hallmark of retinoic acid-induced differentiation of NT2/D1 cells; IFN-γ neither induced neuronal differentiation in the absence of retinoic acid nor affected the appearance of neurons in retinoic acid-treated cultures (data not shown).

Effect of IFN on MHC Antigen Expression. Since IFN-γ induced HLA-A, B, C expression by NT2/D1 cells, we examined in more detail the effect of IFN-γ, and also IFN-α and -β, on MHC antigen expression by several human teratocarcinoma cell lines including 2102Ep cl.4D3, an EC cell line that does not respond to retinoic acid (18, 42), and 14111H, a yolk sac carcinoma line. JAR, a gestational choriocarcinoma cell line, was also studied. Exposure to 100 units/ml of IFN-γ for 1 day was sufficient to induce both HLA-A, B, C and β₂-m expression by NT2/D1 EC and differentiated cells (6 days prior exposure to retinoic acid) and by 2102Ep cl.4D3 EC cells; maximal induction was evident by 2 to 3 days (Fig. 2), and no evidence of IFN-induced differentiation of 2102Ep cl.4D3 cells was observed. Similar, though slightly slower, induction of HLA, A, B, C and β₂-m was also seen in 14111H cultures, but no induction was seen in JAR cells; neither cell line expressed HLA-A, B, C and β₂-m in the absence of IFN-γ. None of the cells expressed HLA-DR antigens, detected by monoclonal antibody DA-2, in the absence or presence of IFN-γ (Fig. 2); nor did IFN-γ alter the growth rate of any of the cells. Since NT2/D1 cultures differentiate more extensively after longer periods in retinoic acid, we also tested cultures treated for 18 days in retinoic acid for inducibility of HLA-DR, but again found no induction (data not shown).

Expression of HLA-A, B, C and β₂-m by NT2/D1 EC cells and their differentiated derivatives is variable in the absence of IFN. In some experiments both antigens are weakly but readily detectable (e.g., Fig. 1); in others they are not detectable. The reason for this variability is unknown (20), but it did not appear to influence the ability of IFN-γ to induce HLA-A, B, C and β₂-m. Further, expression of these MHC antigens changes little as NT2/D1 cells differentiate, although over a series of experiments there is a tendency for a slight reduction in the number of positive cells during the early stages of retinoic acid treatment.
Fig. 1. NT2/D1 EC cells were seeded at 10^6 cells per 75-cm² flask in the presence or absence of 100 units/ml of IFN-γ and 10⁻⁴ M retinoic acid, as indicated. Cultures were harvested after 7 days, and the expression of surface antigens assessed by flow cytometry, except that ME311 and N901 were assayed after 14 days when the greatest change in their expression induced by retinoic acid alone was detected. Non-specific background fluorescence was estimated using P3X63Ag8 as a negative control antibody; the range of the percentage of cells fluorescing after reaction with this antibody was 1 to 5%. L-ALP, liver alkaline phosphatase.

Fig. 2. The effect of IFN-γ on MHC antigen expression by human teratocarcinoma and choriocarcinoma cells. Cells were seeded at 10^6 per 75-cm² flask in medium containing 100 units/ml of IFN-γ; the expression of MHC antigens was assayed by flow cytometry using monoclonal antibodies W6/32 (anti-HLA-A, B, C), B.BM1 (anti-β2-m), and DA-2 (anti-HLA-DR). NT2/D1 (differentiated) cells, obtained by prior growth in the presence of 10⁻⁵ M retinoic acid for 6 days, were reseeded in the absence of retinoic acid. The range of the percentage of cells fluorescing after reaction with the negative control antibody P3X63Ag8 is indicated.

When the teratocarcinoma cells were treated in parallel with IFN-α, -β, or γ (Fig. 4), IFN-γ proved the most effective inducer of HLA-A, B, C and β2-m expression in all the responsive cells. Weak inducing activity of IFN-α and -β for 2102Ep cl.4D3, 1411H, and differentiated NT2/D1 (6 days retinoic acid) cells was observed when present at 100 units/ml. Induction of HLA-A, B, C and β2-m was also seen in NT2/D1 EC culture in the presence of 500 units/ml of IFN-α (data not shown). As in the earlier experiments, JAR cells expressed no MHC antigens under any conditions, even if 500 units/ml of IFN-α, -β, or γ were used. The HeLa derivative, HEp2.5, was included as a control for HLA-DR inducibility (cf. Ref. 43), and this was observed only after exposure to IFN-γ (Fig. 4); in no case, however, was HLA-DR antigen induced in any of the teratocarcinoma or choriocarcinoma cells. Each cell was also tested with a second anti-HLA-DR monoclonal antibody (D1-12), and identical observations were made (data not shown).

Effect of IFN-γ on Murine Teratocarcinoma Cells. Since IFN-γ induces the expression of HLA-A, B, C and β2-m by human EC cells, we also tested whether IFN-γ would induce H-2 expression in murine teratocarcinoma cells. Murine IFN-γ (500...
RESPONSE OF TERATOCARCINOMA CELLS TO IFN

Table 1 Effect of IFN-γ on the expression of H-2, Ia, and embryonic antigens by murine teratocarcinoma cell lines

Cells were seeded at 10⁴ per 75-cm² flask in the absence or presence of 500 units/ml of recombinant murine IFN-γ as shown (FA was only tested with 100 units/ml of IFN-γ). Antigen expression was assayed 4 days later by flow cytofluorimetry; in each case P3X63Ag8 was used as a negative control, and after incubation with this antibody the percentage of cells fluorescing was less than 5%. PCC3/A/1 (59), PCC4azaR (60), and F9 (61) are all EC cell lines derived from 129SV mice (H-2b); PCC4AO is a clonal subline of PCC4azaR identical in all other respects except selected for high resistance to ouabain (62). FA is an EC cell line of AKR (H-2i) origin (25). Morphological examination suggested that PCC4azaR, PCC4AO, F9, and FA cultures (with or without IFN) were composed almost entirely of EC-like cells; PCC3/A/1 cultures, however, contained both EC cells and many morphologically distinct, differentiated cells. F9Ac cl.9 is a retinoic acid-induced differentiated derivative of F9 (45), and resembles PYS, a parietal yolk sac cell line of 129Sv origin (63). KBRSv and LgC57Sv are L2 is an SV40 transformed thyroid cell line of BALB/c origin, and it is inducible for Ia expression by IFN-γ (44).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Presence of IFN-γ</th>
<th>B22-249R1 (anti-H-2Dk)</th>
<th>11-4.1 (anti-H-2Kk)</th>
<th>11-54.3 (anti-Ia)</th>
<th>anti-SSEA-1*</th>
<th>anti-SSEA-3*</th>
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<tr>
<td>PYS</td>
<td>−</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F9Ac1.9</td>
<td>+</td>
<td>49</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F9</td>
<td>−</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>PCC4azaR</td>
<td>+</td>
<td>33</td>
<td>4</td>
<td>5</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>PCC4AO</td>
<td>−</td>
<td>2</td>
<td>1</td>
<td>80</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>PCC3/A/1</td>
<td>+</td>
<td>4</td>
<td>1</td>
<td>93</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>FA</td>
<td>−</td>
<td>46</td>
<td>1</td>
<td>94</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Specificity controls</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KBRSv (H-2)</td>
<td>−</td>
<td>2</td>
<td>28</td>
<td></td>
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<tr>
<td>LgC57Sv (H-2)</td>
<td>−</td>
<td>96</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2 (H-2)</td>
<td>+</td>
<td>29</td>
<td>3</td>
<td>28</td>
<td>5</td>
<td></td>
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</tbody>
</table>

* Murine EC cells, unlike human EC cells, express SSEA-1 (25) but not SSEA-3 (26).

Fig. 4. A comparison of the effects of IFN-α, -β, and -γ on MHC antigen expression by human teratocarcinoma and choriocarcinoma cells; the HeLa-derived HEp2.5 cell line is included as a control for HLA-DR expression (cf. Ref. 43). Cells were seeded as described in the legend to Fig. 2, in the absence or the presence of 100 units/ml of IFN-α, -β, -γ, and assayed after 3 days by flow cytometry with monoclonal antibodies W6/32 (anti-HLA-A, B, C), B.BM1 (anti-β-m), and DA-2 (anti-HLA-DR). No change in cell growth rate was noted in any case (not shown).

units/ml) induced H-2Dk in the parietal yolk sac cell line, PYS, the phenotypically similar line, F9Ac cl.9, derived by differentiation of F9 EC cells induced with retinoic acid, and in cultures of the pluripotent EC line, PCC3/A/1, containing mixtures of EC cells and their derivatives (Table 1). In addition, H-2Dk was weakly induced in the EC line, PCC4azaR, and more strongly induced in a phenotypically identical clonal EC subline, PCC4AO. Induction was weaker, but evident, even at 100 units/ml of IFN-γ (not shown). On the other hand, IFN-γ did not induce the expression of H-2Dk or H-2Kk in the murine EC cell lines, F9 and FA, respectively. As in the human teratocarcinoma lines, Class II MHC antigens were not induced, although they were inducible in the thymoma line, L-2, included as a control (44).

Morphological examination of the cells (not shown) revealed no evidence that IFN-γ induced any differentiation of the EC cells, even of the PCC4AO line which showed the greatest inducibility of H-2 expression. To confirm this, the effect of IFN-γ on the expression of the surface antigens, SSEA-1 and SSEA-3, was determined. Unlike human EC, murine EC cells express SSEA-1 and lose this antigen upon differentiation (24, 45). Further, although murine EC cells do not express SSEA-3 (26), some pathways of differentiation lead to its appearance. Thus, in the experiment shown in Table 1, the small numbers of SSEA-3-positive cells in the cultures of the EC line, FA, probably represent spontaneously differentiated derivatives, while the few SSEA-1-positive (EC) cells in PCC3/A/1 cultures reflected the presence of large numbers of differentiated SSEA-1-negative cells, a few of which were evidently SSEA-3 positive. In no case did the presence of IFN-γ significantly affect the expression of SSEA-1 or SSEA-3, again suggesting no effect of IFN-γ on EC cell differentiation, even in PCC4AO cells.

Antiviral Effects of IFN on Human Teratocarcinoma Cells. To test whether IFN would induce an antiviral state in the panel of human teratocarcinoma cells, the cells were seeded in micro-liter plates and infected with VSV. HEp2.5 cells were used as IFN-responsive controls. Each cell line tested was permissive for VSV, and all the infected cells were killed within 48 h, while the uninfected cultures formed healthy monolayers. Pretreatment with IFN-α, -β, or -γ (up to 4000 units/ml) for 24 h did not protect the cells from VSV infection. These results indicated that the human teratocarcinoma cells did not respond to IFN in the same manner as the murine cells, despite their apparent similarity in morphology and MHC expression.

not protect any of the human teratocarcinoma cells, or JAR cells, from the cytopathic effects of VSV. By contrast, 10 to 20 units/ml of IFN-α, -β, or γ were sufficient to confer significant protection in cultures of HEp2.5 cells, evidenced by the presence of attached cells with healthy morphology, although complete protection, defined by the absence of any viral plaques, required 1000 units/ml. This wide range of response of HEp2.5 cells may reflect the presence of subpopulations of cells with different susceptibilities to IFN in this uncloned line.

To confirm the failure of IFN to protect human teratocarcinoma cells from VSV, supernatants from infected cells, pretreated with IFN-α or -γ, were titered for virus yield using HEp2.5 cells as targets (Fig. 5). Preexposure to 4000 units/ml of IFN-α or IFN-γ lowered the titer of virus produced by HEp2.5 cells by about 300-fold, while preexposure to 250 units/ml of IFN-α or IFN-γ resulted in significant (>10-fold) reduction in virus titer. On the other hand, 4000 units/ml of IFN-α or -γ resulted in no significant changes (<10-fold) in virus production by any of the other cells, with the possible exception of NT2/D1 differentiated cells, derived by growth in retinoic acid for 18 days. In the latter case, IFN-α, but not IFN-γ, at 4000 units/ml, caused a marginally significant reduction (log TCID₅₀, 1.1) in VSV titer; this might indicate the appearance of a subset of cells sensitive to the antiviral effect of IFN-α in the later stages of retinoic acid-induced differentiation.

2-5A Synthetase Induction. The basis for the induction of an antiviral state by IFN is only partially understood, although a number of molecular changes have been associated with this phenomenon. Among these is the induction of the enzyme, 2-5A synthetase, which previous studies have indicated is associated with protection to EMCV and Sindbis virus, but not to VSV. Thus in murine EC cells it was reported that 100 units/ml of IFN induces 2-5A synthetase within 24 h and protection to EMCV and Sindbis virus, but not VSV (11, 14). We therefore analyzed 2-5A synthetase activities in cells grown for 24 h in the presence or absence of IFN-α, -β, or -γ. In the absence of IFN, 2-5A synthetase was detected in control HEp2-5 cells, but not in any of the human teratocarcinoma cells. However, exposure to 100 units/ml of IFN-α, -β, or -γ clearly caused an increase in the activity of this enzyme in HEp2-5 cells, while similar exposure to IFN-α or -β, but not IFN-γ, also resulted in a marked induction of 2-5A synthetase in differentiated NT2/D1 cells (Table 2). On the other hand, no induction was evident in undifferentiated NT2/D1 or 2102Ep cl.4D3 cells, or in 1411H or in JAR cells, under the same conditions. Consistent with the association between 2-5A synthetase and resistance to EMCV and Sindbis, we found that IFN-α and -β, but not IFN-γ, did protect NT2/D1 differentiated cells against the cytopathic effects of these viruses (data not shown).

DISCUSSION

By analogy with murine EC cells, human EC cells, such as NT2/D1 and 2102Ep cl.4D3 cells, are thought to represent malignant counterparts of early embryonic stem cells. On the other hand, the yolk sac carcinoma cell line, 1411H, and the choriocarcinoma cell line, JAR, may be considered malignant counterparts of extraembryonic yolk sac and trophoblast cells, respectively. Yolk sac carcinoma and choriocarcinoma cells are frequent components of human teratocarcinomas, and they probably arise by differentiation from EC stem cells. The differentiation of NT2/D1 cells induced by retinoic acid, however, does not give rise to such extraembryonic cell types, but occurs along somatic lineages (20, 23). Thus, the panel of cells examined in the present study encompasses many of the cell types and differentiation steps found in both the early human embryo and in germ cell tumors. Our results show that these embryonic cells exhibit only a partial response to IFN by comparison with other normal and transformed cells; in the EC cell lines, NT2/D1 and 2102Ep cl.4D3, IFN and, especially, IFN-γ induced Class I MHC antigen expression in the absence of growth inhibition, viral resistance (indicated by lack of protection against VSV infection or induction of 2-5A synthetase), or Class II MHC antigen expression. The differentiation of NT2/D1 cells was accompanied by some changes in responsiveness to IFN; 2-5A synthetase became inducible, an indication of partial resistance (perhaps by a subset of cells) to VSV was noted, and Class I MHC antigen expression was induced to a higher level. On the other hand, 1411H yolk sac carcinoma cells responded to IFN in the same way as the EC cells, while JAR choriocarcinoma cells did not respond to IFN in any way studied. Another gestational choriocarcinoma line, BeWo, has

![Fig. 5. Duplicate microtiter wells were seeded with 10⁴ cells cultured in IFN for 24 h, and infected with VSV, followed by extensive washing. The virus yields were titrated after 2 days in quadruplicate on HEp2.5 cells. The mean log₁₀ TCID₅₀ for the two infected cultures is shown; a difference of 1 log₁₀ unit was considered significant. RA, retinoic acid.](cancerres.aacrjournals.org)
Table 2  Effect of IFN on 2′,5′-olig(A) synthetase activity

<table>
<thead>
<tr>
<th>Cells</th>
<th>IFN*</th>
<th>ATP into 2-5A synthetase (nmol/h/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT2/D1 (differentiated)</td>
<td>No IFN</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>IFN-α</td>
<td>7.69</td>
</tr>
<tr>
<td></td>
<td>IFN-β</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HEp2.5</td>
<td>No IFN</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td>IFN-α</td>
<td>19.61</td>
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<tr>
<td></td>
<td>IFN-β</td>
<td>7.83</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>4.72</td>
</tr>
</tbody>
</table>

* IFN-α, -β, and -γ were all used at 100 units/ml.

been shown to exhibit HLA-A, B, C and β₂-m expression in the presence of IFN-γ (46), and we have confirmed this. The significance of this difference between these two otherwise similar tumor cell lines is unclear, but it is notable that, whereas JAR has never been found to express HLA-A, B, C antigens, BeWo cells variably express low levels seen in the absence of IFN.

In mice, it has been concluded that responsiveness to IFN appears in stages during embryogenesis, and that this developmental regulation is reflected in the response of murine teratocarcinoma cells to IFN. Our results now suggest that, in general, the same is true in humans. However, differences between human and mouse teratocarcinomas are evident. Thus, whereas exposure to IFN does not make the EC cells of either species resistant to VSV, the enzyme 2-5A synthetase is induced in murine EC cells (11), but not in undifferentiated human EC cells. By this criterion human EC cells are less responsive than mouse EC cells, and so they might be considered the more primitive cell type, a concept we have previously suggested on other grounds (15, 16).

The differences with respect to MHC induction are more complex. Human EC cells generally express low levels of MHC Class I antigens (17). On the other hand, it is well established that murine EC cells do not (e.g., Refs. 17, 47–49), although studies of certain somatic cell hybrids of murine EC cells have suggested that the expression of MHC Class I antigens is not necessarily incompatible with expression of an EC phenotype (50, 51). Our present results show that, while human IFN, especially IFN-γ, can induce further expression of these antigens in human EC cells, murine IFN-γ induces them in some murine EC cells. A recent report that F9 EC cells can be induced to express H-2 by IFN-γ (52), in contrast to our observations, may indicate differences between different sublines of F9 comparable to those that we observed in this study between PCC4aza and PCC4AO. Similarly, contrasting results have been obtained by different authors concerning other effects of IFN on murine EC cells. For example, it has been reported that IFN protects (14) or does not protect (53) PCC4 murine EC cells against EMCV, and that double-stranded RNA-dependent protein kinase is inducible (54) or not inducible (11) in different lines of murine EC cells. It has long been known that mouse EC cells, though generally similar to one another, often differ in other properties too. For example, an embryonic antigen expressed by PCC4 EC cells is not expressed by F9 EC cells (55). Such variations might reflect independent changes in gene expression which would result in recognizable cellular differentiation if occurring coordinately, but which may be uncoupled from one another and occur separately in the abnormal setting of a tumor cell without pleiotropic changes in cell phenotype. The observations, that some murine EC cells may express H-2 Class I antigens, albeit in the presence of IFN, and that there is great variability in the expression of HLA-A, B, C antigens by different human EC cells (56), suggest that a distinction between human and mouse EC cells with regard to their differential expression of Class I MHC antigens may not be of fundamental significance.

It has been observed that some murine EC cells form tumors in allogeneic hosts, whereas others do not, and that genetic loci tightly linked and perhaps identical to H-2 Class I loci are involved (e.g., Ref. 57). Our present findings suggest that, in some cases at least, rejection of EC cells by allogeneic hosts may be mediated by H-2 antigens induced by IFN-γ, a product of T-lymphocytes and natural killer cells. Indeed, Ostrand-Rosenberg and Cohan (58) have noted that H-2 antigens are induced in some murine teratocarcinomas following injection into allogeneic hosts; it seems possible that IFN-γ may be a mediator of that phenomenon.

Our present study provides evidence that, as in the mouse, responsiveness to IFNs is developmentally regulated in human teratocarcinoma cells and, by implication, during human embryogenesis, but that even early embryonic cells show a partial response, especially to IFN-γ. Further studies are required to ascertain whether the changes elicited by IFNs have a role in the normal physiology of human development, or whether they are only involved in pathological states of abnormal development. In relation to the former possibility, recent results suggest that IFN-γ may regulate the differentiation of the hemopoietic system by acting in cooperation with other growth factors. We are now investigating this in connection with human EC cell differentiation.

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RESPONSE OF TERATOCARCINOMA CELLS TO IFN


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