Effects of Natural Beta-Interferon and Recombinant Alpha-2B-Interferon on Proliferation, Glucocorticoid Receptor Content, and Antigen Expression in Cultured HL-60 Cells

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In the current study, we investigated the effects of natural beta-interferon (β-IFN) and recombinant alpha-2b-interferon (α-IFN) on the growth of the HL-60 cell line. Cells cultured in a medium that contains various concentrations (from 10 to 1000 IU/ml) of interferons showed a growth inhibition, which reaches the maximum after a 6-day treatment, at the highest dose used. Furthermore, we studied the effect of both β-IFN and α-IFN on the level of glucocorticoid receptors. This was enhanced more than 30% with respect to control in HL-60 cells exposed for 24 hours to concentrations of β-IFN that ranged from 100 to 1000 IU/ml. The increase of the receptor amount was seen even if cells were treated for 5 days, and was not accompanied by a modification of antigen expression of HL-60 cells. α-IFN did not modify the glucocorticoid receptor level substantially in our experimental conditions. Our data indicate that both β-IFN and α-IFN regulate HL-60 cell proliferation. Additional studies are required to clarify if modifications of the receptor level induced by β-IFN could be related to the modulation of hormone-sensitivity in this model.


In the past few years, evidence has accumulated regarding the role of interferon in the regulation of cell proliferation of human cell lines of various histogenesis.1,2 Different degrees of sensitivity to the growth inhibitory action of interferons have been shown by cells in culture, and culture conditions may, in turn, affect cell response to the antiproliferative properties of these drugs.3,4 In addition, interferons promote cell differentiation,5 which, in cells derived from the hemopoietic system, appears to be coupled with the growth arrest in some cases.6

In this context, we reinvestigated the effect of natural beta-interferon (β-IFN) and recombinant alpha-2b-interferon (α-IFN) on HL-60 cell proliferation. In parallel, as in different experimental models β-IFN has been shown to be involved at the same time in the regulation of and is linked to the promotion of cell proliferation in other cases.7 Lymphoid and leukemic cells are reported to be sensitive to alpha-interferon, gamma-interferon, and beta-interferon.1,8,9 Recently, Michalevicz and Revel10 reported that recombinant beta2-interferon inhibits the growth of colonies of normal hemopoietic progenitors, whereas beta2-interferon exerts an antigrowth effect in several leukemic/lymphoma cell lines.11 Controversial results have been reported in regard to the effect of interferons on a human myelogenous cell line (HL-60) established from the peripheral blood of a woman with an acute promyelocytic leukemia.12 Some authors found that these cells are insensitive to human alpha and beta interferon.13 Nevertheless, a combined treatment with dimethylsulfoxide (DMSO) and β-IFN determines a pronounced diminution of HL-60 cell growth associated with an antiferentative effect.13 However, Pan and Guyre14 reported a cytotoxic action by human lymphoblastoid α-IFN in this model.

In this context, we reinvestigated the effect of natural beta-interferon (β-IFN) and recombinant alpha-2b-interferon (α-IFN) on HL-60 cell proliferation. In parallel, as in different experimental models β-IFN has been shown to be involved at the same time in the regulation of
cell growth and modulation of hormone receptor content.\textsuperscript{15-17} we studied the effect of β-IFN and α-IFN on glucocorticoid receptors (GcR), which are present in HL-60 cells.\textsuperscript{18}

\textbf{Materials and Methods}

\textit{Cells}

Light microscopy and cytochemical studies done in our laboratory indicated that HL-60 cells show the same phenotype of the original cell line. They have a diameter of approximately 15 μm, large nucleus and basophilic granules. Furthermore, they show a strong reactivity for peroxidase, Sudan Black B, and naphthol AS-D chloroacetate esterase (data not shown).

The line was maintained in continuous suspension culture in Dulbecco's Modified Eagle's medium (DMEM) (Flow Laboratories, Irvine, UK), supplemented with 20% fetal calf serum (FCS) (Flow Laboratories, Irvine, UK), 100 IU/ml penicillin, and 100 μg/ml streptomycin in the presence of 10% Tryptic Soy Broth (TSB) (Becton Dickinson, Rockville, MD). Cells were subcultured twice a week in 25-cm\textsuperscript{2} sterile tissue culture flasks.

\textit{Interferon}

The specific activity of B-IFN was $10^7$ IU/mg of protein. The specific activity of α-IFN was $2 \times 10^8$ IU/mg of protein. Both β-IFN and α-IFN were dissolved in culture medium at the appropriate concentrations before use.

\textit{Immunologic Marker Study of HL-60 Cells}

To detect immunologic markers in our HL-60 cells, we used the following monoclonal antibodies (MoAb): OKT11 MoAb (CD-2, E-receptor) and OKDR MoAb (anti-HLA-DR, MHC class II antigen) (Ortho Diagnostic System Inc., Raritan, NJ); HPCA-1 MoAb (CD-34, antilymehoblasts and CFU-C); Mol MoAb (CD-11b, antimonocytes-granulocytes); MY9 MoAb (CD-33, antimonocytes-granulocytes and CFU-C); MY4 MoAb (CD-14, antimonocytes) and B4 MoAb (CD-19, pan-B) (Coulter Immunology, Hialeah, FL).

Phenotypic analysis was done by indirect immunofluorescence staining as reported previously\textsuperscript{19} using a fluorescein isothiocyanate labeled goat-antimouse IgG (Ortho FITC—GAM reagent). Fluorescence was measured using the Ortho Spectrum III flow cytometer (Ortho-Diagnostic System Inc., Westwood, MD).

\textit{Cell Growth Experiments}

HL-60 cells were seeded in DMEM, supplemented as reported previously, at the initial density of 150,000 cells/ml in 35 mm plastic Petri dishes. In experimental dishes β-IFN and α-IFN were added at the following concentrations: 10, 100, and 1000 IU/ml of medium. Cell growth was evaluated by counting the cells in a hemocytometer after 3 and 6 days of exposure to the drug. Cell viability was studied by trypan blue dye exclusion. Each experiment was done in triplicate.

\textit{Glucocorticoid Receptor Assay}

The number of specific GcR sites in control cells and in cells treated for different periods of time with 10, 100, and 1000 IU of β-IFN/ml of medium was determined using a whole cell assay.\textsuperscript{20} Briefly, cells were washed in medium without FCS and TSB, centrifuged at 200 × g, and resuspended in the same medium. Aliquots (0.1 ml) of cell suspension (approximately $2 \times 10^6$ cells) were incubated in triplicate with a saturating concentration (30 nM) of $^3$H-dexamethasone, ($^3$H-dex) (specific activity, 49.9 Ci/mmol; DuPont/NEN Research Products, Dreieich, West Germany) in the presence or absence of a 200-fold unlabeled hormone (Sigma Chemical Co., St. Louis, MO) excess. For Scatchard analysis, cells were incubated with a concentration of $^3$H-dex from 1.2 to 30 nM. After the incubation (60 minutes at 37°C under 5% CO$_2$ in air), 1.5 ml of Hanks' balanced salt solution (HBSS) was added to each tube. The tubes were kept at room temperature for 5 minutes and then centrifuged at 200 × g for 5 minutes. The washing process was repeated twice. Pellets were suspended in 1 ml of 80% ethanol overnight, and the suspensions were transferred to counting vials. In order to determine the exact number of cells recovered at the end of the washing procedure, four additional tubes that contained the same amounts of cells were prepared and processed in the same manner as the tubes used for binding determinations. Radioactivity was counted in ethanol extracts. Specific binding was calculated as the difference between the values obtained with and without the excess of radioinert steroid. Binding capacity was expressed as the number of sites per cell. The evaluation of binding data by Scatchard analysis was done as described previously.\textsuperscript{20}

\textit{Data Analysis}

Analysis of variance associated with the Dunnett's test for a control versus several treatments was done to establish the statistical significance of the data.\textsuperscript{21}

\textbf{Results}

\textit{Immunologic Characterization of HL-60 Before and After β-IFN}

Results obtained using MoAb to detect the immunologic markers indicate that B4 MoAb (CD-19) and OKT11...
TABLE 1. Reactivity of Monoclonal Antibodies With HL-60 Cell Line

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Specificity</th>
<th>IFN IU/ml (3rd day of treatment)*</th>
<th>IFN IU/ml (6th day of treatment)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>MY9</td>
<td>CD-33</td>
<td>x</td>
<td>58.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>29.82</td>
</tr>
<tr>
<td>MY7</td>
<td>CD-13</td>
<td>x</td>
<td>77.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>15.71</td>
</tr>
<tr>
<td>Mo1</td>
<td>CD-11b</td>
<td>x</td>
<td>8.30</td>
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<td></td>
<td></td>
<td>SD</td>
<td>2.42</td>
</tr>
<tr>
<td>HPCA1</td>
<td>CD-34</td>
<td>x</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>5.02</td>
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<tr>
<td>MY4</td>
<td>CD-14</td>
<td>x</td>
<td>4.23</td>
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<tr>
<td></td>
<td></td>
<td>SD</td>
<td>0.06</td>
</tr>
<tr>
<td>OKDR</td>
<td>HLA-DR</td>
<td>x</td>
<td>4.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>4.56</td>
</tr>
<tr>
<td>B4</td>
<td>CD-19</td>
<td>x</td>
<td>2.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>0.38</td>
</tr>
<tr>
<td>OKT11</td>
<td>CD-2</td>
<td>x</td>
<td>10.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>8.95</td>
</tr>
</tbody>
</table>

MoAb: monoclonal antibody; B-IFN: beta-interferon; SD: standard deviation.

Results are expressed as the percentage of cells fluorescent above background in an indirect immunofluorescence assay with the indicated monoclonal antibody. Each value represents the mean (+ SD) of three experiments. A positive reaction was defined as a minimum of 20% of cells stained.

* Cultured in absence or in presence of different concentrations of 6-IFN.
† P < 0.05 (Dunnett's test of the control versus each one treatment).

MoAb (CD-2), which identify lymphocytes and were used to evaluate our cell lineage fidelity, react weakly. Furthermore, our cells show a MY7 and MY9 strong expression and MY4 and HLA-DR weak expression (Table 1). Our results concur with the data by Griffin et al., who reported that HL-60 cell line shows weak fluorescence staining with MY4 MoAb (CD-14) and stronger reactivity of MY7 MoAb (CD-13). Concerning MY9 MoAb (CD-33), it reacts with immature myeloid cells but not with lymphocytes, platelets, or erythrocytes. Finally HLA-DR is reported to be expressed weakly in HL-60 cells. Based on these data, we concluded that our HL-60 possess the characteristics of the original cell line and are of promyelocytic nature.

When HL-60 cells are exposed for 3 or 6 days to the action of different concentrations of 6-IFN, it seems that they do not show any significant modification of their phenotypic characteristics. The analysis of variance of the data, followed by the Dunnett's test to compare the control with each one of the treatments, does not show any significant difference in most of the markers studied at the different concentrations of 6-IFN used (Table 1).

MY4 (CD-14) and Mo1 (CD-11b) are expressed in untreated and treated cells in a low percentage of elements, indicating that neither have nor acquired monocyte characteristics.

In regard to MY9 (CD-33), this antigen is normally expressed by leukemia cell lines and still remains positive in our treated cells. The variations seen do not affect the positivity of the staining.

Effect of Interferons on HL-60 Cell Proliferation

Treatment of HL-60 cells with 10 to 100 IU/ml of 6-IFN determines a weak inhibition of cell proliferation after 3 days (10.5% to 17.7% with respect to control, P < 0.05 and P < 0.01, respectively; Dunnett's test) and reaches 27% at the highest concentration (1000 IU/ml, P < 0.01). After a 6-day treatment the inhibition of cell proliferation is enhanced (19.6% with respect to control at 10 IU/ml, P < 0.01; 29.4% at 100 IU/ml, P < 0.01; and about 37% at 1000 IU/ml, P < 0.01; Dunnett's test) (Fig. 1). Figure 2 shows the effect of a-IFN on HL-60 cell proliferation after 3 and 6 days of treatment. a-IFN produces a diminution of cell growth that reaches 25% with respect to the control.
to control \( (P < 0.01, \text{Dunnett's test}) \) on day 3 at the highest concentration. If the exposure to \( \alpha \)-IFN is prolonged to day 6, the reduction of cell growth ranges from 24% to 31.7% with respect to control \( (P < 0.01; \text{Dunnett's test}) \).

These results suggest that both \( \beta \)-IFN and \( \alpha \)-IFN are effective in the inhibition of HL-60 cell growth in our culture conditions and do not concur with the data reported by Hamburger et al., who showed that \( \alpha \)-IFN does not influence HL-60 cell proliferation unless these cells are pretreated with DMSO.

**Effect of Interferons on GcR Level in HL-60 Cells**

In regard to GcR, after a 24-hour \( \beta \)-IFN treatment, no modification of receptor level is seen at the lowest concentration of \( \beta \)-IFN used \( (10 \text{ IU/ml}) \). At the dose of 100 to 1000 IU/ml of \( \beta \)-IFN, receptor level is enhanced more than 30% with respect to control \( (P < 0.05; \text{Dunnett's test}) \) (Fig. 3). The enhancement of receptor level is still seen after 5 days of \( \beta \)-IFN treatment at the highest concentration used \( \text{(data not shown)} \).

Figure 4 shows a typical Scatchard plot of binding data related to GcR modification produced by 1000 IU \( \beta \)-IFN/ml of medium on day 5 of treatment.

The \( K_d \) of GcR \( (9.4 \times 10^{-9} \text{ M}) \) is not modified by \( \beta \)-IFN treatment \( (11.6 \times 10^{-9} \text{ M}) \), indicating that the increase seen in the GcR level is not due to a reduction of the binding affinity. There is not a significant difference between the two slopes \( \text{(i.e., the two lines are parallel)} \) which indicates that \( \beta \)-IFN only induces an increase of the total measurable number of the binding sites.

After 1, 3, and 5 days of treatment, \( \alpha \)-IFN at the concentrations tested produces some variations of receptor content, which does not reach the statistical significance \( \text{(Dunnett's test)} \) (Table 2).

**Discussion**

Our data indicate that both \( \beta \)-IFN and \( \alpha \)-IFN regulate HL-60 cell proliferation. The results concerning \( \alpha \)-IFN are in agreement with the data of Pan and Guyre. In regard to the inhibitory action of \( \beta \)-IFN, the disagreement with previous reports could probably be explained by the different culture conditions or the higher degree of purification of \( \beta \)-IFN used in our experiments. We observed the reduction of cell proliferation irrespective of the presence of 20% of FCS in the culture medium.

Furthermore, \( \beta \)-IFN enhances the GcR level after a brief treatment, whereas \( \alpha \)-IFN induces some enhancement of these receptors even if the difference between treated and untreated cells does not reach the level of significance. In this case, \( \beta \)-IFN exerts an action similar to that displayed on other steroid receptors, such as estrogen and progesterone receptors in human mammary cancer cell lines and in human endometrial adenocarci-

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**Fig. 2.** Effect of \( \alpha \)-IFN on growth of HL-60 cells. HL-60 cells were treated with \( \alpha \)-IFN at the concentration indicated for 3 and 6 days. Columns represent the average of three determinations, bars represent the SD; the number at the column bottom is the percentage of growth inhibition with respect to the control. \( * = P < 0.05; ** = P < 0.01; \) Dunnett's test of the control versus each one treatment.

**Fig. 3.** Effect of \( \beta \)-IFN on GcR concentrations in HL-60 cells, after a 24-hour treatment. Columns represent the average of three determinations, bars represent the SD; the number at the column bottom is the percentage of GcR increase with respect to the control. \( * = P < 0.05; \) Dunnett's test of the control versus each one treatment.

**Fig. 4.** Scatchard analysis of \(^3\)H-dex binding in HL-60 cells untreated \( \text{(ctr)} \) or treated with \( \beta \)-IFN \( (1000 \text{ IU/ml}) \) for 5 days. The \( K_d \) (dissociation constant) in untreated and treated cells are \( \text{of } 9.4220E-09 \) and \( 11.6331E-09 \), respectively. The \( r \) (correlation coefficients for straight lines) before and after \( \beta \)-IFN treatment are 0.85 and 0.93, respectively.
The GcR content of the lymphoid cells increases as the tumor. Furthermore, beta2-interferon has been reported to confirm a maturation induced by α-IFN in HL-60 cells. Thus, the GcR increase shown in HL-60 cells exposed to IFN have an additive or synergistic inhibitory effect at the cellular level. Additional studies are needed to establish if it is possible to achieve the same receptor enhancement with α-IFN that was obtained with β-IFN using different doses or modalities of treatment. Recently, Pan and Guyre showed that glucocorticoids combined with IFN have an additive or synergistic inhibitory effect on the growth of several leukemic cell lines. These authors suggest that their findings could be explained as a potentiating effect of dexamethasone on the antiproliferative activity of IFN. Based on our results, it is possible to hypothesize that the GcR modulation by IFN in HL-60 cells represents the biochemical basis of the enhanced responsiveness to glucocorticoid. Resistance to glucocorticoid in HL-60 cells, even in the presence of specific binding sites, may be attributed to a defective receptor mechanism that could be affected by IFN theoretically. Our findings, if confirmed in vivo, could create new perspectives on the role of interferon in the management of human leukemia, even in neoplasias less responsive or unresponsive to glucocorticoids.

**REFERENCES**


### Table 2. Effect of α-IFN on GcR Level in HL-60 Cells

<table>
<thead>
<tr>
<th>α-IFN concentration (IU/ml)</th>
<th>Sites/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day of treatment</td>
</tr>
<tr>
<td>0</td>
<td>7728.13 ± 1656.34*</td>
</tr>
<tr>
<td>10</td>
<td>9039.27 ± 3154.74</td>
</tr>
<tr>
<td>100</td>
<td>7687.07 ± 1455.66</td>
</tr>
<tr>
<td>1000</td>
<td>7673.03 ± 2623.01</td>
</tr>
</tbody>
</table>

* Values represent the mean ± SD from three different experiments.

α-IFN: alpha-interferon; GcR: glucocorticoid receptor.


28. Cidlowski JA, Cidlowski NB. Glucocorticoid receptors and cell cycle: Evidence that the accumulation of glucocorticoid receptors during the S phase of the cell cycle is dependent on ribonucleic acid and protein synthesis. *Endocrinology* 1982; 110:1635-1662.