Effect of Natural Beta-Interferon on Cell Proliferation and Steroid Receptor Level in Human Breast Cancer Cells

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The effect of natural beta-interferon (β-IFN) on cell proliferation and steroid receptor level was investigated in CG-5 human breast cancer cell line. β-Interferon determines an appreciable diminution of cell growth, at concentrations ranging from 100 to 1000 IU/ml, which is enhanced when serum content of the culture medium is lowered. Low concentrations of β-IFN (10–100 IU/ml) produce, after a 5-day treatment, an increase in estrogen receptors (ER) and progesterone receptors (PR). No variation of ER and PR levels is observed when β-IFN is added directly to the cell homogenate before the assay. Our data suggest that β-IFN could affect hormone sensitivity through a modification of ER and PR in neoplastic mammary cells.


IN RECENT YEARS much attention has been focused on the potential anticancer properties of interferons, a group of proteins originally identified as antiviral agents. In fact, in addition to the activation of the immune system, interferons are able to influence gene expression and cell growth and differentiation.1

In particular natural beta-interferon (β-IFN) has been shown to have a pronounced antiproliferative activity over a broad range of histologically different solid tumors.2–6 Moreover it is a clinically useful drug for its relative safety in man.7,8

The mechanism by which β-IFN regulates human cell growth is not completely known, even though it has been observed that it causes a prolonged intermitotic interval in cultured human fibroblasts and the arrest in G0–G1 phase of the cell cycle in the Burkitt’s lymphoma derived Daudi cell line.9,10

As far as breast cancer is concerned, β-IFN has recently been shown to affect another biological parameter: subsequent to a short-period treatment, it produces an increase of hormone receptors for estrogen (ER) and progesterone (PR) in tumor biopsy specimens from patients with advanced disease.11 Similar results were obtained in vitro by Dimitrov et al., who found an enhancement of estrogen binding activity, adding alpha-IFN to cell homogenate of breast cancer tissue.12

These reports in addition to the few data available concerning the antiproliferative activity of β-IFN on cultured human mammary neoplastic cells,2,13 led us to investigate on the effect of β-IFN on the cell growth and on the possible modulation of ER and PR in a human breast cancer cell line, named CG-5.

CG-5 cells are a variant of the MCF-7 cell line, characterized by a high degree of estrogen responsiveness and an appreciable content of estrogen, androgen, glucocorticoid, and progesterone receptors.14

The data presented in this report indicate that β-IFN inhibits cell proliferation and determines an increase of ER and PR levels in CG-5 cells, opening new perspectives in using this drug in the management of hormone-dependent breast cancer.

Materials and Methods

Culture of Cells

CG-5 cells were obtained in 1982 in the Laboratory of Molecular Endocrinology of the Catholic University of Sacred Heart, Rome, Italy.

They are derived from a casual contamination by MCF-7 cells of a primary culture of a pleural effusion from a patient with advanced breast cancer. Established CG-5 cell cultures show characteristic arrangement of cells to form duct-like structures and dome formations.

The cells show a low degree of growth in the absence of estrogen, whereas, in the presence of 10⁻⁸ M estradiol, they show a two-fold increase of the cell number over
FIG. 1. Effect of various concentrations of β-IFN on the growth of CG-5 cells, cultured in medium supplemented with 10% FCS. The absolute number of cells/dish in control cultures was 510,000 after 3 days and 2,240,000 after 6 days. Bars represent standard error. *P < 0.05; **P < 0.01; ***P < 0.001.

Antiproliferative Assays

Cells at logarithmic growth phase were trypsinized and plated out at a density of 50,000 cells/ml into 60-mm plastic Petri dishes in DMEM supplemented as described above.

Twenty-four hours after plating, by which time cells became attached to the surface of the dish, medium was replaced with fresh medium containing ten-fold dilutions of β-IFN, varying from 10 to 1000 IU/ml. Triplicate cultures were set up for each drug concentration. Control dishes (without β-IFN) were cultured in parallel. Cell counts were performed after 3 and 6 days of exposure to the drug.

The influence of growth factors and steroid molecules present in FCS on the sensitivity of cells to β-IFN also was investigated. In this case CG-5 cells were cultured in DMEM supplemented with 5% FCS or 5% charcoal-treated FCS and the same experimental procedure described above was followed. Each test was repeated at least three times to assess the reproducibility of the results.

Estrogen Receptor Assays

Whole cell assay: Cells were plated at a density of 50,000 cells/ml into 60-mm plastic Petri dishes in DMEM supplemented with 10% FCS. Twenty-four hours later medium was renewed and the cells were exposed to varying concentrations of β-IFN ranging from 10 to 1000 IU/ml, but control cells did receive no treatment. After 5 days of exposure to β-IFN, ER determination was performed. Cells were washed twice with phosphate-buffered saline (PBS), pH 7.4, at 37°C and incubated in a serum-free DMEM. Two hours later medium was renewed with the same type of medium plus (2,4,6,7-3H)17-β-estradiol (3H-E2) (90.4 Ci/mmol) (New England Nuclear, Boston, MA) at a concentration of 5 nM. Additional dishes were filled with medium with the same concentration of 3H-E2 and a 100-fold molar excess of diethylstilbestrol (DES) (Sigma) to assess non specific binding. After 1 hour incubation at 37°C, cells were washed three times with PBS at room temperature and then disrupted in 1 mol/l of sodium hydroxide (NaOH) overnight. Aliquots were transferred to scintillation vials for radioactivity counting. In each experiment additional dishes were run in parallel to each treatment for cell number determination. Values reported in Table 3 represent the mean of three different determinations.

Binding sites were calculated from the difference of incorporated radioactivity after incubating in the absence or presence of an excess of unlabeled steroid. Binding data were then referred to the number of viable cells in plates processed in the same manner of those used for binding determinations.
Cytosol assay: Cells grown in standard conditions at logarithmic growth phase were detached from the culture dish with a rubber policeman, washed twice with cold PBS, pH 7.4, and homogenized in a buffer of this composition: 10 mmol/l Tris, 1.5 mmol/l EDTA, 10 mmol/l monothioglycerol, 10% glycerol, at pH 7.4.

Cell homogenate was divided in aliquots which were added with varying doses of β-IFN ranging from 10 to 1000 IU/ml and immediately centrifuged at 4°C for 30 minutes at 100,000 × g. The supernatants were then incubated with 3H-E2 5 nmol/l ± a 100-fold excess of unlabeled DES. After an 18-hour incubation at 4°C a suspension of dextran-coated charcoal16 was added. Tubes were then incubated for 10 minutes at 4°C. The mixture was centrifuged for 10 minutes at 2000 × g and aliquots of supernatants were transferred to scintillation vials for counting.

Protein concentration was determined by the method of Bradford17 using bovine serum albumin (Sigma) as a standard.

Progesterone Receptor Assays

Whole cell assay: The same experimental procedure of ER whole-cell assay was followed. Incubation was performed in a serum-free DMEM containing 16α-Ethyl-21-hydroxy-19-nor(6,7-3H)pregn-4 en-3,20-dione (3H-ORG 2058, 49 Ci/mmol, Amersham International, Buckinghamshire, England) 5 nmol/l ± a 200-fold excess of unlabeled ORG 2058.

Cytosol assay: The same experimental procedure of ER cytosol assay was followed. Cytosols were incubated with 3H-ORG 2058 5 nmol/l ± a 200-fold excess of unlabeled ORG 2058.

Results

Effect of β-IFN on Cell Number

Figure 1 shows the effect of various concentrations of β-IFN (10–1000 IU/ml) on the growth of CG-5 cells cultured in medium supplemented with 10% FCS. After 3 days of exposure to the drug a slight inhibition of cell proliferation respect to control is seen at the highest dose (21%, P < 0.01), whereas, after 6 days of treatment this inhibition is observed starting from the concentration of 100 IU/ml of β-IFN (32%, P < 0.001) and it is very pronounced at 1000 IU/ml (52%, P < 0.001). No change was observed in the inhibition magnitude if medium was renewed every 3 days (data not shown).

Figure 2 shows the effect of concentrations of β-IFN above indicated on the growth of CG-5 cells cultured in medium supplemented with 5% FCS. In this case the inhibition of cell proliferation is evident after 3 days of exposure to the drug starting from the concentration of 100 IU/ml of β-IFN (21%, P < 0.005).

Moreover, the inhibition obtained using 1000 IU/ml of β-IFN in these conditions (31.4%, P < 0.001) is increased if compared to that observed when the percentage of the serum present in the medium was higher.

After 6 days of treatment with β-IFN a small inhibition of cell proliferation also is seen at the concentration of 10 IU/ml of β-IFN (21% compared with control, P < 0.001) and it increases significantly when the drug concentration is augmented (31%, P < 0.001 at 100 IU/ml; 63%, P < 0.001 at 1000 IU/ml).

If cells are cultured in the presence of medium supplemented with 5% charcoal-treated FCS, no further enhancement in the inhibition is seen, as depicted in Figure 3.

Effect of β-IFN on ER and PR Level

Table 1 and 2 illustrate the effect on ER and PR levels of various concentrations of β-IFN added to the cell homogenate (cytosol assay). No significant variation was observed at any concentration of β-IFN tested. These data suggest that β-IFN per se does not interfere in estrogen and progesterone binding to their cytoplasmic

<table>
<thead>
<tr>
<th>IFN concentration (IU/ml)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
<th>Experiment 5</th>
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<tbody>
<tr>
<td>0</td>
<td>31.5*</td>
<td>41.2</td>
<td>37.2</td>
<td>68.0</td>
<td>21.2</td>
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<tr>
<td>10</td>
<td>38.8</td>
<td>34.8</td>
<td>37.4</td>
<td>45.1</td>
<td>18.7</td>
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<td>100</td>
<td>43.2</td>
<td>26.4</td>
<td>34.3</td>
<td>46.6</td>
<td>28.7</td>
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<td>1000</td>
<td>36.9</td>
<td>40.1</td>
<td>40.9</td>
<td>47.1</td>
<td>21.2</td>
</tr>
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</table>

ER: estrogen receptors; β-IFN: beta-interferon; IFN: interferon.

* fmole/mg protein.
The first conclusion which can be drawn from the examination of the effect of \( \beta \)-IFN on CG-5 cell growth is that this cell line is sensitive to the interferon growth inhibitory action. In fact, about 30% inhibition of cell proliferation of attached cells in the presence of 100 IU/ml of \( \beta \)-IFN and about 45% in the presence of 1000 IU/ml of \( \beta \)-IFN, when medium is supplemented with 10% FCS, is observed.

More stringent culture conditions (presence in the medium of 5% FCS) determine a higher inhibition (more than 60% at the concentration of 1000 IU/ml). This supports the idea that factors probably antagonizing interferon action are present in the serum, as recently suggested by Inglot. For this reason, the diminution of serum percentage, present in the culture medium, and, consequently, the decreased amount of these factors, enables interferon to act without any interference.

Moreover, antagonizing factors are not removed by charcoal treatment. In fact, using stripped serum, no further enhancement of the inhibition of cell proliferation is observed compared to that seen when the same percentage (5%) of untreated serum is used.

The peculiar characteristics of CG-5 cells which are estrogen-sensitive, justified the attention devoted to the variation of the receptor content after interferon treatment.

The exposure of CG-5 cells even at low doses of \( \beta \)-IFN (10–100 IU/ml) produces an enhancement of ER and PR, evaluated by a whole-cell assay, after 5 days. The reason why high concentrations of the drug (1000 IU/ml) does not modify ER level, whereas, the enhancement of PR content results dose-related, is still unclear and requires further investigation.

Nevertheless, our data underline the different biological properties of \( \beta \)-IFN compared to \( \alpha \) and \( \gamma \)-IFN. In fact, treatment with high doses of \( \gamma \)-IFN (500 IU/ml) does not affect ER content in ZR-75-1 cells, as demonstrated by Marth et al., whereas the addition of \( \alpha \)-IFN to the homogenate of breast cancer specimens seems to enhance the receptor level, as shown by Dimitrov and co-workers.

The finding that \( \beta \)-IFN inhibits cell proliferation and, on the other hand, determines an enhancement of ER and PR is not surprising. In fact, it is widely documented that interferons influence cellular physiology and behavior in different ways, causing biochemical modifications, which are often opposite. With regard to this, it is known that interferon strongly inhibits total protein synthesis, but it was also found to induce several mRNAs and the corresponding

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**Table 2. PR Level After the Addition of Different Concentrations of \( \beta \)-IFN to CG-5 Cell Homogenate**

<table>
<thead>
<tr>
<th>IFN concentration (IU/ml)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>53.9*</td>
<td>54.6</td>
<td>77.2</td>
<td>44.1</td>
<td>88.4</td>
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<tr>
<td>10</td>
<td>61.6</td>
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<td>75.9</td>
<td>30.0</td>
<td>81.4</td>
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<tr>
<td>100</td>
<td>69.7</td>
<td>47.8</td>
<td>65.9</td>
<td>28.5</td>
<td>87.1</td>
</tr>
<tr>
<td>1000</td>
<td>78.2</td>
<td>48.5</td>
<td>78.8</td>
<td>21.1</td>
<td>72.2</td>
</tr>
</tbody>
</table>

PR: progesterone receptors; \( \beta \)-IFN: beta-interferon; IFN: interferon.

* Sites/cell.

**Table 4. PR Level in CG-5 Cells Exposed for 5 Days to \( \beta \)-IFN**

<table>
<thead>
<tr>
<th>IFN concentration (IU/ml)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
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<tr>
<td>0</td>
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<td>24,080</td>
<td>31,790</td>
<td>28,328</td>
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<tr>
<td>10</td>
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<td>37,732</td>
<td>30,937</td>
<td>36,649</td>
<td>29,097</td>
<td>30,305†</td>
</tr>
<tr>
<td>100</td>
<td>55,688</td>
<td>69,867</td>
<td>33,199</td>
<td>41,277</td>
<td>36,208</td>
<td>35,578‡</td>
</tr>
<tr>
<td>1000</td>
<td>69,650</td>
<td>52,326</td>
<td>45,420</td>
<td>33,691</td>
<td>35,758</td>
<td>33,693‡</td>
</tr>
</tbody>
</table>

PR: progesterone receptors; \( \beta \)-IFN: beta-interferon; IFN: interferon.

* Sites/cell.

† Not significant.

‡ \( P < 0.05 \) (Wilcoxon’s signed rank test).
proteins.\textsuperscript{22} In addition, the functions, the identities and the relationships of these proteins to some enzymes whose level is increased by interferon treatment remain to be established.\textsuperscript{22}

The observed lack of parallelism between the effect displayed by \(\beta\)-IFN on cell growth and the modifications produced on steroid receptor level (which are not necessarily correlated) could have some relevant implications from the clinical point of view, if one thinks that the response to some agents used in the therapy of breast cancer is correlated to the receptor level.\textsuperscript{23}

If \(\beta\)-IFN could determine also \textit{in vivo} an increase of ER and PR, as very preliminary data from Pouillart \textit{et al.}\textsuperscript{11} seem to indicate, it could make patients more responsive to endocrine manipulations, through potentiating receptorial content of neoplasias.

Further investigations are needed to establish the doses and the modalities of the interferon treatment which could \textit{in vivo} be effective in achieving the same receptor enhancement obtained \textit{in vitro}. Nevertheless, it is encouraging that the concentration of \(\beta\)-IFN required to modify \textit{in vitro} ER and PR is very low.

\begin{thebibliography}{99}