Interferon Enhances the Expression of Epstein–Barr Virus Early Antigen in Daudi Cells

By MICHAEL G. TOVEY,* MICHEL DRON AND ION GRESSER

Institut de Recherches Scientifiques sur le Cancer, Laboratory of Viral Oncology, BP8–94802 Villejuif, France

(Accepted 21 December 1981)

SUMMARY

Treatment of the Burkitt lymphoma-derived cell line Daudi with highly purified human interferon-α (IFN-α) increased up to 100-fold the number of cells expressing Epstein–Barr virus (EBV)-determined early antigen (EA) without inducing the synthesis of virus capsid antigen (VCA), a late virus function. The increase in the number of EA-positive cells was proportional to interferon concentration up to 10^4 international units (IU)/ml. Treatment of Daudi cells with the same number of units of either partially purified (sp. act. 10^6 IU/mg protein) or electrophoretically pure (sp. act. 2 × 10^8 IU/mg protein) IFN-α both gave a similar increase in EA expression, strongly suggesting that the effects observed were indeed due to interferon. Furthermore, treatment of relatively interferon-insensitive Raji cells with IFN-α (1 to 10^4 IU/ml) had no significant effect on either spontaneous or 5-iodo-2′-deoxyuridine (IdUrd)-induced expression of EA or VCA. Human IFN-β also enhanced the expression of EBV EA in Daudi cells. In contrast, when the latent EBV present in Daudi cells was activated to enter into a replicative cycle, either by induction with IdUrd or by superinfection with the non-transforming P3HR1 strain of EBV, then treatment with human IFN-α caused a marked inhibition of EA expression. Our results suggest that interferon can exert a differential action on virus replication depending upon the state of the virus genome within the cell.

INTRODUCTION

Epstein–Barr virus (EBV) is a lymphotropic herpesvirus which infects the majority of mankind, causing infectious mononucleosis when infection is delayed until adolescence or adulthood (De Thé, 1980). EBV is also associated with two human tumours, Burkitt’s lymphoma (BL) and nasopharyngeal carcinoma (De Thé, 1980). Lymphoid cell lines of B cell origin can be established from patients with these diseases and from lymphocytes transformed in vitro by EBV (Henle et al., 1967). In most lymphoid cells the lytic virus cycle is usually repressed even though the cells carry multiple copies of the EBV genome. These cells therefore provide a valuable model for the study of virus latency.

The interferon response has been shown to play an important role in the limitation of virus infections (Gresser et al., 1976a, b). Interferons also affect cell division and cell function, influence the immune response and exhibit a marked anti-tumour action (Gresser & Tovey, 1978; Stewart, 1979). In many instances in which latent virus is activated, interferon is also produced (Tovey, 1980), suggesting that interferon may play a role in the control of virus activation and/or the limitation of subsequent virus replication. It was therefore of interest to determine the effect of interferon on the expression of the latent EBV genome in human lymphoid cells. We report here that treatment of the human BL cell line Daudi, which is sensitive to both the anticellular and antiviral actions of interferon (Adams et al., 1975), with...
human interferon (either IFN-α or IFN-β) enhanced the expression of EBV-determined early antigen (EA).

**METHODS**

*Cell culture.* Lymphoblastoid cells were cultivated in RPMI 1640 medium with 10% foetal calf serum (Gibco) in static suspension culture.

*Chemicals.* 5-Iodo-2’-deoxyuridine (IdUrd) was purchased from Sigma.

*Immunofluorescence.* EBV EA or virus capsid antigen (VCA) was determined on 50000 acetone-fixed cells either by direct immunofluorescence with fluorescein isothiocyanate (FITC)-conjugated EA (+) VCA (+) or EA (−) VCA (+) human sera, or by indirect immunofluorescence with EBV-specific human sera and FITC-conjugated goat anti-human IgG (Huntington Laboratories, Huntington, Ind., U.S.A.).

The comparative titres of the EA (+) VCA (+) serum were 640 and 2560 respectively, and those of the EA (−) and VCA (+) serum were <5 and 640. The specificity of the immunofluorescence was verified by the use of a control EBV-negative human serum (EA titre <5, VCA titre <5). When the percentage of positive cells was low (<0.5%) and/or the differences between samples were small, all the positive cells on a slide were counted, or a differential count was performed on at least 1500 cells per slide. Results are expressed as the mean of four replicates per sample.

*Interferon preparations.* Human IFN-α was prepared from peripheral blood leukocytes or from the human lymphoid cell line Namalwa as described previously (Mogensen & Cantell, 1977). Two types of preparations were used in this study. Partially purified IFN-α of specific activity 10⁶ international units (IU)/mg protein and highly purified IFN-α which had been purified by affinity chromatography (Mogensen & Cantell, 1979) to a specific activity of 2 × 10⁸ IU/mg protein. This interferon was electrophoretically pure when subjected to electrophoresis in polyacrylamide gels containing SDS (Mogensen et al., 1981). Partially purified human IFN-β of specific activity 2 × 10⁶ IU/mg protein was prepared from the human diploid fibroblast cell line FS4 (Havell & Vilček, 1972).

**RESULTS**

The effect of human IFN-α on the expression of EBV antigens was studied in three human Burkitt lymphoma-derived cell lines, Daudi, P3HR1 and Raji, all of which harbour multiple copies of the EBV genome, and which exhibit high, intermediate and low sensitivity respectively to both the antiviral and anticellular actions of interferon (Adams et al., 1975).

The producer cell line Daudi contains a small percentage of cells (<0.1%) in which the latent EBV 'spontaneously' becomes activated as shown by the presence of cells which express both EBV-determined EA and VCA. In all experiments, treatment of Daudi cells with human IFN-α increased the number of cells expressing EBV EA. In most experiments interferon treatment caused a 3- to 5-fold increase in the number of EA-positive cells (Table 1), while in some experiments a 30- to 100-fold increase was obtained, giving up to 10% EA-positive cells (Table 1). Although the factors governing the degree of the response to interferon have not been established, variations in cell proliferation can influence virus induction (Hampar, 1979), and may account for the differences observed in the extent of EA enhancement in interferon-treated cultures. However, a similar increase in the number of EA-positive cells was obtained when either exponentially multiplying or stationary phase cells were treated with IFN-α (data not shown).

Treatment of Daudi cells for 2 to 4 h with IFN-α, followed by incubation in medium without interferon, was sufficient to give a maximum increase in the number of EA-positive cells determined at 24 h, although no significant increase in EA expression was observed 6 h after the start of interferon treatment. The number of EA-positive cells, in both untreated and
Table 1. Effect of interferon on the expression of EBV-determined antigens in Daudi cells*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Treatment</th>
<th>Specific activity (IU/mg protein)</th>
<th>EA-positive cells (%) ± s.D.</th>
<th>VCA-positive cells (%) ± s.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>Untreated</td>
<td>1.12 ± 0.1</td>
<td>0.22 ± 0.12</td>
<td>3.10 ± 0.06 P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Partially purified IFN-α</td>
<td>1 × 10⁶</td>
<td>3.10 ± 0.06 P &lt; 0.001</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Partially purified IFN-α</td>
<td>1 × 10⁶</td>
<td>144 ± 17</td>
<td>0.10</td>
</tr>
<tr>
<td>3‡</td>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Highly purified IFN-α</td>
<td>2 × 10³</td>
<td>28.5 ± 3.5</td>
<td>31 ± 0.16</td>
</tr>
<tr>
<td>4</td>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Partially purified IFN-α</td>
<td>1 × 10⁶</td>
<td>4.0 ± 0.1 P &lt; 0.001</td>
<td>0.12 ± 0.11 NS</td>
</tr>
<tr>
<td></td>
<td>Highly purified IFN-α</td>
<td>1 × 10⁸</td>
<td>4.5 ± 1.3 NS</td>
<td>0.20 ± 0.10 NS</td>
</tr>
<tr>
<td>5</td>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Partially purified IFN-β</td>
<td>2 × 10⁶</td>
<td>0.3 ± 0.1 P &lt; 0.001</td>
<td>0.10 ± 0.03</td>
</tr>
</tbody>
</table>

* Daudi cells were cultivated in either medium alone or medium containing 10⁴ IU/ml of the interferon preparation indicated. Human IFN-α was prepared from peripheral blood leukocytes except in experiment 3 where the IFN-α used was prepared from Namalwa cells. The number of EA-positive cells was then determined at 24 h by indirect immunofluorescence as described in Methods.

† Mean of four individual experiments with four separate determinations per experiment.
‡ Determined by Student’s t-test.
§ NS, Not significant.
‖ Determined at 72 h.
Fig. 1. Daudi cells were cultivated in either medium alone or medium containing the concentration of partially purified interferon indicated. The number of EA-positive cells was then determined at 24 h by indirect immunofluorescence as described in Methods.

Table 2. Effect of IFN-α on the induction of EA in Daudi cells*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IFN concn. (IU/ml)</th>
<th>EA-positive cells (%) ± s.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>–</td>
<td>0.08</td>
</tr>
<tr>
<td>IdUrd</td>
<td>–</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>IdUrd + IFN-α (pretreatment)</td>
<td>10^2</td>
<td>3.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>10^4</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>IdUrd + IFN-α (simultaneous treatment)</td>
<td>10^2</td>
<td>4.4 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>10^4</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>IdUrd + IFN-α (continuous treatment)</td>
<td>10^2</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>10^4</td>
<td>1.7 ± 0.4</td>
</tr>
</tbody>
</table>

* Daudi cells were cultivated in static suspension culture in either medium alone or medium containing highly purified IFN-α (sp. act. 10^8 IU/mg protein) prepared from peripheral blood leukocytes. After 24 h incubation at 37 °C, IdUrd (25 μg/ml) was added to each culture except the untreated control, and IdUrd plus interferon was added to one culture which had previously been incubated in medium alone. The number of EA-positive cells was then determined at 48 h by indirect immunofluorescence as described in Methods. (At 48 h, cell viability was >95% in all cultures.)

Table 3. Effect of IFN-α on the expression of EBV-determined antigens following superinfection of Daudi cells with P3HR1 virus*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EA-positive cells (%) ± s.D.</th>
<th>VCA-positive cells (%) ± s.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.04 ± 0.01</td>
<td>0.01 ± 0.005</td>
</tr>
<tr>
<td>P3HR1 virus</td>
<td>12.5 ± 1.5</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>P3HR1 virus + IFN-α (pretreatment)</td>
<td>5.6 ± 0.3</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>P3HR1 virus + IFN-α (simultaneous treatment)</td>
<td>5.8 ± 0.6</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>P3HR1 virus + IFN-α (continuous treatment)</td>
<td>2.5 ± 0.3</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

* Daudi cells (10^6) which had been preincubated for 24 h in either medium alone or medium containing 10^4 IU/ml of highly purified interferon (sp. act. 10^6 IU/mg protein) prepared from peripheral blood leukocytes were resuspended in 10 ml of nutrient medium containing P3HR1 virus at an m.o.i. of approx. 1. After a 1 h adsorption period at 37 °C the cells were centrifuged (1000 rev/min for 5 min) and resuspended at 5 × 10^5 cells/ml in either medium alone or medium containing 10^4 IU/ml of highly purified IFN-α. The number of EA- or VCA-positive cells was then determined at 48 h by indirect immunofluorescence as described in Methods. (At 48 h, cell viability was >95% in all cultures.)
interferon-treated cultures, decreased progressively with time from the maximum reached at 24 h (Table 1). Treatment of Daudi cells with IFN-β also increased markedly the number of cells expressing EBV EA (Table 1). Again, the increase in the number of EA-positive cells was maximal after 24 h treatment.

A similar increase in the number of EA-positive cells was obtained when Daudi cells were treated with the same number of units of either partially purified (sp. act. 10^6 IU/mg protein) or electrophoretically pure (sp. act. 2 × 10^8 IU/mg protein) human IFN-α (Table 1). The increase in the number of EA-positive Daudi cells was proportional to interferon concentration over the range of 1 to 10^4 IU/ml both for partially purified (Fig. 1) and highly purified IFN-α preparations (data not shown).

Treatment of Daudi cells with IdUrd or sodium butyrate results in the induction of approx. 5 to 10% EA-positive cells (Hampar et al., 1972; Gerber, 1972; Lucka et al., 1979). Pretreatment of Daudi cells for 24 h with 10^4 IU of human IFN-α markedly inhibited the number of EA-positive cells observed after induction with IdUrd (Table 2). Treatment of Daudi cells with interferon at the same time as IdUrd also inhibited EA expression (Table 2), although to a lesser extent than when cells were pretreated with interferon. Combined pretreatment and treatment of Daudi cells with interferon caused a greater inhibition of EA induction than either treatment alone (Table 2). Interferon treatment also inhibited EA expression in Daudi cells treated with sodium butyrate (data not shown).

Superinfection of Daudi cells with the non-transforming P3HR1 strain of EBV induced an abortive virus cycle in 10 to 20% of the cells, characterized by the production of both EA and VCA. Treatment of Daudi cells with human IFN-α, either prior to or following superinfection, inhibited the induction of both EA and VCA (Table 3).

Treatment of P3HR1 cells with IFN-α at concentrations ranging from 1 to 10^4 IU/ml had no significant effect on either the spontaneous or IdUrd-induced expression of EA or VCA (Table 4). Similarly, IFN-α had no significant effect on the expression of EBV-determined antigens in Raji cells (Table 4).
We have shown that both human α- and β-interferons stimulate the spontaneous expression of EBV EA in the Burkitt lymphoma-derived cell line Daudi. Thus, treatment of Daudi cells with IFN-α increased 3- to 100-fold the number of cells expressing EBV EA. To our knowledge, these results show for the first time that interferon can activate the genome of a latent human virus resulting in the production of virus-specified proteins. Although interferon has been shown to increase the intracellular concentration of the viral P30 group-specific antigen in cells infected with endogenous murine leukaemia virus (Friedman & Ramseur, 1974; Billiau et al., 1974), the P30 antigen accumulates within the cell as a result of the inhibition by interferon of a late stage in virus assembly and/or release (Friedman, 1979) and may not be comparable to the activation of latent EBV.

Although interferon treatment consistently enhanced EA expression in Daudi cells, no effect was observed on the production of VCA which is spontaneously expressed in a low percentage of cells (≤0.01%). The productive virus cycle in Daudi cells has been reported to be abortive (Ernberg & Klein, 1979), and consistent with this observation we have been unable to detect any infectious virus in cultures of untreated or interferon-treated Daudi cells.

The results of experiments showing that treatment of Daudi cells with the same number of units of either partially purified (sp. act. 10^6 IU/mg protein) or electrophoretically pure (sp. act. 2 x 10^8 IU/mg protein) IFN-α induced a comparable increase in EA expression indicate that interferon was the factor responsible for the effects observed. Furthermore, interferon treatment only enhanced EA expression in highly interferon-sensitive Daudi cells but not in the much less sensitive P3HR1 cells or in relatively interferon-resistant Raji cells.

Interferon can exert apparently quite different effects on EBV depending upon the type of infection established by the virus. Thus, interferon treatment resulted in activation of the virus genome in Daudi cells latently infected with EBV, whereas the same treatment inhibited virus replication in Daudi cells superinfected with the non-transforming P3HR1 strain of EBV, or induced with IdUrd or sodium butyrate. Adams et al. (1975) also reported that interferon did not inhibit spontaneous EA expression in Daudi cells even though the same treatment inhibited EA expression following virus activation. Thus, the effect of interferon on EBV replication may depend upon the physical state of the virus DNA within the cell. In cells latently infected with EBV the majority of virus DNA is in a free circular form, although some virus DNA is integrated into the host's chromosomes (Adams, 1979). Mozes & Defendi (1979) have shown that in simian virus 40-infected cells integrated virus genomes are resistant to the action of interferon, while within the same cell non-integrated virus genomes remain interferon-sensitive.

Although the mechanisms of the interferon-induced increase in EA expression are unknown, it seems unlikely that this effect was secondary to inhibition of cell proliferation since an increase in EA was observed after 2 to 4 h of interferon treatment. Furthermore, a comparable effect was observed in interferon-treated stationary phase cells. Interferon has been shown to enhance the expression of a number of cellular antigens including histocompatibility antigens and β-2 microglobulin (Lindahl et al., 1973, 1974; Fellous et al., 1979). This effect is also observed within a few hours of treatment and is probably due to an increased synthesis of the antigen (Fellous et al., 1981).

In many instances in which latent virus is activated, interferon is also produced, e.g. in a graft versus host reaction (Hirsch et al., 1973; Wu et al., 1975), in an in vitro allogenic reaction (Olding et al., 1975; Green et al., 1969), or when lymphoid cells are treated with chemical inducers or mitogens (Epstein, 1976; Hampar et al., 1972; Gerber, 1972; Lucka et al., 1979; Moroni et al., 1975; Greenberg et al., 1975; Tovey et al., 1977, 1979). It seems unlikely, however, that the activated virus induces interferon, since we have shown that in the EBV-negative human lymphoid cell line BJAB treated with IdUrd (Tovey et al., 1977),

DISCUSSION
Interferon enhances EBV early antigen

interferon production can occur in the absence of virus activation. Nevertheless, the close association between virus activation and interferon production suggests that interferon may play a role in the control of virus activation and/or the limitation of subsequent virus replication.

It may seem paradoxical that interferon which inhibits virus multiplication should actually activate a latent virus. However, the interferon-induced expression of virus antigens may render these latently infected cells susceptible to host defence mechanisms. For example, the natural killer sensitivity of Daudi cells has been shown to increase in parallel with EA expression following activation of the latent EBV genome (Blazar et al., 1980). Thus, the expression of virus-induced antigens in conjunction with the interferon response may be determining factors in the host’s ability to mount an effective antiviral response against latently infected cells.

We thank Dr Erik Mogensen for the gift of the partially purified and highly purified human IFN-α, Dr Hochkeppel for the gift of the partially purified human IFN β, Dr Gilbert Lenoir for the gift of the EBV-specific human sera, and Miss Jacqueline Begon-Lours for skilled technical assistance. This work was supported in part by grants from DGRST (contract no. 930-469) and DRET (contract no. 78-34-210 and 80-34-522).

REFERENCES


(Received 21 October 1981)