Epstein–Barr Virus Replication in Interferon-treated Cells

By NIGEL A. SHARP, JOHN R. ARRAND and MICHAEL J. CLEMENS

1Division of Biochemistry, Department of Cellular and Molecular Sciences, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE and
2Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, U.K.

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SUMMARY

The Burkitt's lymphoma-derived cell line Daudi is latently infected with the Epstein–Barr virus (EBV). These cells are very sensitive to the growth inhibitory and differentiation-inducing effects of human interferon-α (IFN) and we have examined the possibility that these responses are due to induction of EBV replication. Our results indicate that only a very small proportion of cells (<0.3%) are induced by IFN treatment to express EBV lytic genes at the RNA or protein level, whereas cell growth inhibition is complete under the same conditions. In contrast, when EBV replication is chemically induced the large increase in lytic gene transcripts, including that of the BZLF1 trans-activator gene, is partially inhibited by concomitant IFN treatment. The increase in viral DNA copy number in chemically induced Daudi cells is also partially inhibited by IFN but no effect of IFN on the level of viral DNA is observed in uninduced cells.

The interferons (IFNs) are a family of proteins with antiviral, antiproliferative and immunoregulatory activities (Clemens & McNurlan, 1985; Pestka et al., 1987). IFN treatment of B lymphocytes has been shown partially to prevent infection by Epstein–Barr virus (EBV) (Menezes et al., 1976; Lvovsky et al., 1981; Doetsch et al., 1981), but the effect of IFN on spontaneous and chemically induced EBV replication in latently infected cell lines is less clear. Viral replication can be induced by treatment of such cells with chemical agents such as 12-O-tetradecanoylphorbol 13-acetate (TPA) plus sodium butyrate (Bauer, 1983), after which the cells express viral early antigens (EA), membrane antigens (MA) and viral capsid antigens (VCA).

The Burkitt's lymphoma-derived cell line Daudi is one of the most sensitive to the antiproliferative action of IFN (Adams et al., 1975; Gewert et al., 1981). The mechanism by which IFN mediates growth inhibition and ultimately impairs cell viability is not fully understood, but IFN has pleiotropic effects on Daudi cells including induction of B cell differentiation (Exley et al., 1987).

Two groups have reported that IFN can induce EBV replication in Daudi and other cell lines: Tovey et al. (1982) found that spontaneous EA expression in Daudi cells could be enhanced up to 100-fold by IFN (giving 14% positive cells), and Zeng et al. (1982) reported that IFN treatment of B95-8 cells also increased the number of cells expressing EA/VCA from 1% to 8%. Such induced replication of EBV in latently infected Daudi cells would seem paradoxical for an antiviral agent. However, viral replication could be related to IFN-induced growth inhibition and differentiation because the expression of viral replicative antigens in a cell is incompatible with continued cellular proliferation (Gergely et al., 1971) and has been associated with B cell differentiation (Crawford & Ando, 1986). Furthermore, agents that induce EBV replication such as TPA and sodium butyrate can also act as differentiation inducers in some cell types. We have therefore investigated the possibility that cell growth regulation by IFN in this system may be related to the induction of the viral lytic cycle.

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Table 1. Effect of IFN on spontaneous and induced EBV antigen expression*

<table>
<thead>
<tr>
<th>Cells</th>
<th>Antigen</th>
<th>Positive cells (%)</th>
<th>Stimulation (-fold)</th>
<th>Positive cells (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>+ IFN</td>
<td>Induced</td>
<td>Induced + IFN</td>
</tr>
<tr>
<td>Daudi</td>
<td>EA/VCA</td>
<td>0.10</td>
<td>0.16</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>EA/VCA</td>
<td>0.005 ± 0.006</td>
<td>0.042 ± 0.007†‡</td>
<td>5.3</td>
<td>46.9</td>
</tr>
<tr>
<td></td>
<td>EA/VCA</td>
<td>0.014 ± 0.009</td>
<td>0.017 ± 0.012</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EA/VCA</td>
<td>0.12 ± 0.02</td>
<td>0.27 ± 0.02‡</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EA/VCA</td>
<td>0.019 ± 0.005</td>
<td>0.078 ± 0.006††</td>
<td>4.1</td>
<td>34.8 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>EA/VCA</td>
<td>0.042 ± 0.012</td>
<td>0.096 ± 0.028†‡</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EA(D)</td>
<td>0.017 ± 0.007</td>
<td>0.096 ± 0.028†‡</td>
<td>1.8</td>
<td>36.6 ± 1.7</td>
</tr>
<tr>
<td>Daudi</td>
<td>MA</td>
<td>0</td>
<td>0.02</td>
<td>-</td>
<td>9.7 ± 1.8</td>
</tr>
<tr>
<td>B95-8</td>
<td>EA/VCA</td>
<td>0.36</td>
<td>0.29</td>
<td>0.8</td>
<td>33</td>
</tr>
<tr>
<td>B95-8</td>
<td>EA/VCA</td>
<td>0.22 ± 0.04</td>
<td>0.39 ± 0.12</td>
<td>1.8</td>
<td>44</td>
</tr>
<tr>
<td>Raji</td>
<td>EA/VCA</td>
<td>0.03</td>
<td>0.03</td>
<td>1.0</td>
<td>51</td>
</tr>
</tbody>
</table>

* The EBV-positive cell lines Daudi, B95-8 and Raji, propagated as described previously (Gewert et al., 1981), were incubated in the absence (control) or presence of 100 units/ml IFN (Daudi) or 1000 units/ml IFN (B95-8 and Raji). Where indicated, EBV replication was induced by the addition of 20 ng/ml TPA and 3 mm-sodium butyrate (Bauer, 1983). The percentage of cells expressing EBV replicative antigens was determined after 2 days by indirect immunofluorescence as described by Bauer (1983) using either EA/VCA-positive human sera or monoclonal antibody R3 against EA(D) (Pearson et al., 1983), or 72A1 against MA (Hoffman et al., 1980). Results are expressed as percentage positive cells ± standard deviation. The statistical significances were determined using Student's t-test (†, P < 0.05; ††, P < 0.01; †††, P < 0.001). Preparations of IFN-α were generously supplied by Wellcome Biotechnology (Beckenham, U.K.). Recombinant IFN-α2 was a gift from Professor D. C. Burke (University of East Anglia, Norwich, U.K.).

Using indirect immunofluorescence the proportion of EA/VCA-positive control Daudi cells was always observed to be low (less than 0.15%) (Table 1). In all experiments, there was a small increase in the percentage of positive cells after IFN treatment but this was not always statistically significant. Such a stimulation is seen when either naturally produced or recombinant IFN-α is used indicating that IFN-α, rather than some minor contaminant, is indeed responsible for this small effect. In the six experiments shown, the mean stimulation by IFN of spontaneous EA/VCA expression in Daudi cells was 2.8 ± 1.5-fold which is significantly different from 1 (P < 0.05). Monoclonal antibodies confirmed that control and IFN-treated Daudi cells express EA, but no expression of the late antigen MA could be detected.

In this series of experiments there was some correlation between the extent of the small increase in EA/VCA-positive cells and the degree of inhibition of Daudi cell growth by IFN (data not shown). However, the growth of the majority of the cells could clearly be inhibited in the absence of any activation of EBV antigen expression.

A similar small increase in the percentage of EA/VCA-positive Daudi cells could also be obtained following treatment with 10 ng/ml TPA [which acts as a differentiation inducer (Ho et al., 1987; Schaffer et al., 1988)] or by growing the cells to a terminal density of approximately 1·6 × 10^6/ml (1.9- and 3.3-fold stimulations respectively; not shown). These results suggest that the increase in EA/VCA-positive Daudi cells after IFN treatment could be a consequence of differentiation and/or the cessation of cell growth rather than a specific action of IFN per se.

When Daudi cells were treated with TPA and sodium butyrate a large increase in the number of cells expressing the replicative antigens was observed (up to 5000-fold) (Table 1). This increase was mainly due to EA-expressing cells, with about 25% of these cells progressing through the viral cycle to express MA. Concomitant treatment with lymphoblastoid or recombinant IFN-α at 100 units/ml resulted in a reduction of the number of cells supporting viral replication by 24 to 60%. IFN blocked the appearance of EA(D) and MA equally as demonstrated using monoclonal antibodies (Table 1), showing that IFN was not acting only at a late stage in the replicative cycle.

B95-8 cells have been reported to give up to 8% EA/VCA-positive cells after IFN treatment (Zeng et al., 1982), but in the experiments described in Table 1 no more than 0.4% positive cells were seen. There was no significant difference between the values for IFN-treated and control...
Fig. 1. The effects of IFN on spontaneous and induced EBV transcripts. Daudi cells were incubated in the absence or presence of 100 units/ml IFN for 2 days, with or without induction of the EBV productive cycle by 20 ng/ml TPA and 3 mM-sodium butyrate. Total cytoplasmic RNA was prepared essentially as described by Schaffer et al. (1988). RNAs (15 μg) were denatured, electrophoresed on a 1% agarose gel containing formaldehyde and transferred to nylon membranes as described by Maniatis et al. (1982). Cloned restriction fragments of EBV DNA (Arrand et al., 1981) were labelled with [α-32P]dCTP using random priming (Feinberg & Vogelstein, 1984) and hybridized to the Northern blots (Maniatis et al., 1982). Probes used were BamHI M (a), EcoRI E (b), BamHI Z (c) or cellular α-actin cDNA (Minty et al., 1981) (d). The membranes were washed to a final stringency of 0.1 x SSC at 65 °C. The autoradiographs of the membranes are shown, with the sizes of the RNAs given in kb. The lanes contained RNAs prepared from Daudi cells treated as follows: (1) control, (2) IFN, (3) induced, (4) induced plus IFN. Actin RNA levels were similar in the induced and induced plus IFN RNA preparations (d).

cells. After induction by TPA/sodium butyrate a large increase in the percentage of fluorescent cells was observed and this increase was partially blocked by IFN (27 to 41% inhibition), indicating that this marmoset cell line is capable of responding to human IFN. Raji cells likewise showed no stimulation of spontaneous EA expression by IFN (Table 1). After induction more than 51% of the cells became EA-positive and this was slightly reduced by IFN, in contrast to the results of Zeng et al. (1982) who found a further stimulation of EA expression.

Northern blotting was used to investigate the mechanism whereby IFN mediates a reduction in the percentage of EA/VCA-positive Daudi cells after induction of EBV replication. The BamHI M region of the EBV genome encodes several proteins of the EA(D) complex (Pearson et al., 1983; Cho et al., 1985; Sample et al., 1986) including one encoded by the BMRFL open reading frame (Baer et al., 1984) which is recognized by the anti-EA(D) monoclonal antibody used above. The EcoRI E region encodes seven late and one early mRNA (Farrell, 1987). One of the late mRNAs is from BcLF1 which encodes the major component of VCA (Vroman et al., 1985; Davison & Scott, 1986). Fig. 1 shows the results of experiments using probes corresponding to these regions of the genome using RNAs from a single experiment.

In uninduced Daudi cells there is a very low level of a 2-3 kb RNA from BamHI M (Fig. 1a). Three major RNA species of 3-4, 2-3 and 1-6 kb are detected in induced Daudi cells and these are reduced in amount by 17-5, 24 and 34%, respectively in IFN-treated cells. The 2-3 kb RNA is transcribed from BMRFL (Sample et al., 1986); the 3-4 kb RNA has been shown to encode a ribonucleotide reductase from BarF1 (Gibson et al., 1984).

The EcoRI E probe shows extensive transcription from this region of the viral genome in induced Daudi cells (Fig. 1b) in agreement with the findings of others (Hummel & Kieff, 1982). The largest band of 4-0 kb is probably from BcLF1. Densitometry showed that this RNA was reduced in IFN-treated induced Daudi cells by 55%.

The switch to the lytic cycle of EBV replication is brought about by trans-activating proteins encoded by the BamHI Z, M and R regions. The BZLF1 gene is probably analogous to the immediate early genes of herpes simplex virus types 1 and 2 (Biggin et al., 1987). Since the expression of BZLF1 is critical in determining whether EBV in a particular cell remains latent or enters the replicative cycle, it was of interest to compare the level of its expression in control
and IFN-treated cells. Northern blotting revealed no detectable expression from this region of the genome in control or IFN-treated uninduced Daudi cells but after induction of EBV replication two RNAs of 3.1 and 1.1 kb were detected (Fig. 1c). These RNAs were reduced in abundance by 24 and 41% respectively by concomitant IFN treatment of the cells. The 1.1 kb RNA encodes BZLF1 and the 3.1 kb RNA contains both the BRLF1 and BZLF1 open reading frames (Biggin et al., 1987). Since all later events in the viral cycle are dependent on the expression of the trans-activator genes, our results are consistent with the possibility that the reduction by IFN of the percentage of EA/VCA-positive Daudi cells after induction of EBV replication is due to a decrease in the proportion of cells in which BZLF1 is induced.

Southern blotting was used to examine the EBV DNA content of control and IFN-treated Daudi cells that had been incubated in the presence or absence of TPA/sodium butyrate to induce viral replication. IFN had no effect on the level of the EcoRI E EBV DNA fragment in uninduced Daudi cells (Fig. 2). After induction of viral replication the level of EBV DNA was increased more than sixfold. This increase in DNA content was partially blocked in the presence of IFN since in this case the increase was only fourfold. The 40% reduction by IFN of EBV DNA accumulation in the lytic state is larger than the 28% reduction in the number of cells expressing EA/VCA in this experiment. This suggests that in addition to reducing the number of Daudi cells in which EBV is induced to replicate, IFN may partially block lytic EBV DNA accumulation even in EA/VCA-positive cells. This could be a consequence of the inhibition of synthesis of early lytic cycle proteins.

Overall, our results indicate that IFN does not produce a physiologically significant induction of EBV replication in Daudi cells and indeed inhibits such induction by other agents. IFN treatment of uninduced Daudi cells does cause a small increase in EA/VCA expression and there is a tendency for the largest stimulations to occur in cell populations that show the greatest inhibition of cell growth. However, the important point is that following IFN treatment growth inhibition occurs in virtually all of the cells in the culture whereas induction of EBV replication is restricted to less than 0.3% of the cells. Furthermore, in some experiments cell growth could be inhibited without any statistically significant elevation of antigen-expressing cells at all. We can thus rule out the possibility that IFN mediates growth inhibition in Daudi cells through a virus-induced cytopathic effect.

The results described here differ in some respects from those of Tovey et al. (1982) and Zeng et al. (1982) who reported IFN-induced increases in EBV antigen expression in the Daudi, B95-8 and Raji cell lines; the reasons for these discrepancies are not clear. Tovey et al. reported up to 100-fold induction of EA in Daudi cells by IFN but only found three- to fivefold increases in most experiments; the latter effect is comparable with the results described here. However, their experiments used up to 10000 units/ml of IFN preparations which could have contained minor contaminants, such as other cytokines. Neither Tovey et al. nor Zeng et al. conclusively proved that their EBV-inducing factor was indeed IFN (e.g. by use of recombinant IFN or anti-IFN
neutralizing antibodies). Other groups have also failed to find any induction of EBV replication after IFN treatment of these and other cell lines (Adams et al., 1975; Lvovsky et al., 1981; Takimoto et al., 1985).

The IFN-mediated reduction in the number of chemically induced cells expressing early and late viral replicative antigens has been observed by several groups (Adams et al., 1975; Lvovsky et al., 1981; Tovey et al., 1982; Takimoto et al., 1985). We have investigated this for the first time at the RNA level and have shown that IFN reduces the levels of RNAs from the BamHI M and EcoRI E regions of the EBV genome. These results indicate that the reduction in the percentage of EA/VCA-positive induced Daudi cells by IFN treatment cannot be ascribed entirely to inhibition of viral protein synthesis at the level of translation; rather, the impairment of BZLF1 expression in IFN-treated cells most likely leads to decreased trans-activation of EBV lytic cycle genes at the transcriptional level.

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REFERENCES


Short communication


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