Spontaneous Interferon Production and Epstein–Barr Virus Antigen Expression in Human Lymphoid Cell Lines

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SUMMARY

Established human lymphoid cell lines, many of which spontaneously produce interferon, differ in the efficiency by which they allow expression of Epstein–Barr virus (EBV) lytic functions. Six EBV carrying lymphoid cell lines, selected to either be extremely susceptible or very refractory to EBV superinfection, were tested for spontaneous interferon production. Only the three cell lines which were poorly superinfectable with EBV were found to produce interferon. These same three lines could not be induced to express EBV-specific early antigens from intrinsic EBV genomes. It is suggested that interferon acts as a negative control factor affecting a cell's susceptibility to EBV.

INTRODUCTION

Established lymphoid cell lines of human origin differ greatly in their susceptibility to Epstein–Barr virus (EBV) superinfection (Klein, Dombos & Gothoskar, 1972; Adams & Klein, 1973) and in the degree to which the intrinsic EBV genomes carried by these cells are expressed (Klein & Dombos, 1973). Some of these cell lines also spontaneously produce interferon (Swart & Young, 1969; Zajac, Henle & Henle, 1969) which could be a factor influencing virus gene expression. In the accompanying paper (Adams, Strander & Cantell, 1975), interferon was shown to suppress the early antigen (EA) synthesis that follows either EBV superinfection or 5'-iododeoxyuridine (IdU) induction of the Daudi cell line. On the basis of these results, it seemed likely that spontaneous interferon production could indeed influence the phenotype of a lymphoid cell line with regard to EBV gene expression. Here six cell lines, selected either to be very susceptible or resistant to EBV superinfection, were tested for spontaneous interferon production.

METHODS

Cell lines and reagents. The origins of 5 of the 6 lymphoid cell lines studied here are listed in the accompanying paper (Adams et al. 1975). The sixth line, JHTC-33, was established from a patient with infectious mononucleosis (Junge, Hoekstra & Deinhardt, 1971). Other reagents were the same as described in the accompanying paper (Adams et al. 1975).

Spontaneous interferon production. Conditions similar to those described by Zajac et al. (1969) were used to maximize spontaneous interferon yields. Centrifugally sedimented cells were resuspended at a concentration of 5 to \(10^6\) cells/ml in RPMI 1640 medium supple-
mented with only 2% foetal bovine serum (FCS) and incubated for 24 h at 37 °C. The supernatant medium was collected by centrifuging after the first 24 h incubation period and saved at 4 °C. The cell pellet was resuspended in an initial volume of fresh medium containing 2% serum and incubated for the additional 24 h period. The second supernatant fluid was likewise collected, combined with the first 24 h supernatant fluid, filtered through a 0.45 μm Millipore filter and stored at 4 °C. Prior to testing for antiviral activity, the serum concentration of all preparations was increased to 15% which was the concentration of foetal bovine serum employed to grow the Daudi cells used in the interferon assay.

Interferon assay. Interferon was titred on Daudi cells by determining its inhibitory effect on the expression of EA after EBV superinfection of these cells. The assay, which was developed to measure anti-EBV activities, is relatively rapid and in model experiments was found to reliably detect as little as one unit/ml of standard human leukocyte interferon. The assay was performed in a vol. of 200 μl in a 96 well microplate (Falcon Microtest II or Linbro IS-FB-96-TC) as follows: 50 μl of virus diluted in 15% medium containing serum was mixed with 50 μl of cells (10⁶ living cells/ml) directly in a microwell and the plates incubated for 30 min at 37 °C. One hundred μl of 15% medium containing FCS was then added, and the incubation was continued for two days at 37 °C in a humidified CO₂ incubator. At the end of the 48 h incubation period, about 75% of the supernatant medium was removed from each microwell. The cells were resuspended in the remaining medium and the contents of individual wells layered on top of 1 ml phosphate buffered saline (PBS) in small plastic precipitation tubes and the cells sedimented at 1000 g. After removal of the PBS wash liquid, the cell pellet, which is sufficient for a single smear, was transferred to a microscope slide and air dried. The acetone fixed smears were stained with a FITC conjugated EA-positive serum and the numbers of antigen positive cells enumerated (Adams & Klein, 1973). The cells were either pre-treated with interferon and washed free of interferon prior to addition of EBV or, alternatively, in some tests twice the concentration of interferon was added to untreated Daudi cells in the 100 μl of medium which was added after virus adsorption. All interferon units are expressed in terms of the reference research standard 69/19 (National Institute of Medical Research, London).

Interferon inactivation tests. Trypsinization: an equal volume of a 0.25% solution of trypsin in PBS was added to the interferon preparation in 2% medium containing serum and samples incubated for 1 h at 37 °C. After incubation any remaining trypsin was inactivated by addition of soy bean trypsin inhibitor and the serum concentration was raised to 15%. Heat inactivation: samples in RPMI medium supplemented with 15% serum were heated in a 60 °C water bath. Acid treatment: samples were titrated to pH 2 with 1 N-HCl and kept for 2 to 4 days at 4 °C before being back titrated with an equal molar amount of NaOH. DNase digestion: pancreatic DNase was added together with MgCl₂ to a final concentration of 50 μg/ml and 1 mM respectively and the samples were incubated for 30 min at 37 °C. RNase digestion: pancreatic RNase was added to a concentration of 2 μg/ml and samples incubated for 30 min at 37 °C. Sedimentability: sedimentation at 100000 g was performed at 4 °C in the SW 50.1 rotor of a Spinco preparative centrifuge. Dialysability: dialysis was overnight at 4 °C against RPMI 1640 medium supplemented with antibiotics but no serum.

RESULTS

Spontaneous interferon production of lymphoid cell lines

Three readily superinfectable lymphoid cell lines, all of which could also be induced with IdU to express intrinsic EBV gene functions, and three non-inducible and very poorly
Table 1. *Spontaneous interferon production of different lymphoid cell lines*  

<table>
<thead>
<tr>
<th>Production of spontaneous interferon (units/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Superinfectable, inducible lines</td>
<td></td>
</tr>
<tr>
<td>Daudi</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Raji</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>NC-37</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Poorly superinfectable, non-inducible lines</td>
<td></td>
</tr>
<tr>
<td>Namalwa</td>
<td>50±20</td>
</tr>
<tr>
<td>Akuba</td>
<td>40±20</td>
</tr>
<tr>
<td>JHTC-33</td>
<td>20±10</td>
</tr>
</tbody>
</table>

* The conditions used for preparing spontaneous interferon are described in Methods. At least two cell supernatant preparations from each cell line were prepared and tested on Daudi cells at 1:2 and 1:4 dilutions for their effect on the EA expression that follows EBV superinfection. The apparent interferon concentration of the undiluted preparation was calculated by comparing the reduction in antigen positive cells to that observed with known amounts of partially purified human leukocyte interferon. Three different cell supernatant fluids from the three poorly superinfectable lines were assayed and the spread in results indicates the range between apparent interferon concentrations of the different preparations.

Table 2. *Characterization of the anti-EBV activities spontaneously produced by various lymphoid cell lines*  

<table>
<thead>
<tr>
<th>Source of antiviral preparation</th>
<th>Daudi</th>
<th>Namalwa</th>
<th>Akuba</th>
<th>JHTC-33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human leukocytes</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Trypsin</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>RNase</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>DNase</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Dialysis (overnight)</td>
<td>100</td>
<td>95</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>60 °C, 1 h</td>
<td>90</td>
<td>75</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>pH 2</td>
<td>100</td>
<td>95</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>None</td>
<td>105</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Raji†</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>

* The apparent interferon titre of the various preparations before and after the different treatments was determined as described in Table 1. The effect of the various treatments on the antiviral activity are expressed as percent interferon activity remaining after treatment to that present in the untreated preparation.

† Instead of pre-treating the Daudi cells with interferon prior to superinfection, the Daudi cells were first infected with EBV and after virus adsorption had occurred the cells were exposed to the various antiviral preparations.

‡ Percent activity remaining was calculated from the level of EA-positive Raji cells using the Daudi standard interferon dose response curve and assuming that untreated Daudi and Raji cells are equally susceptible to EBV superinfection.

superinfectable cell lines were tested for spontaneous interferon production (Table 1). The Daudi, Raji and NC-37 lines are three of the most easily EBV superinfectable lines available (Adams & Klein, 1973). None of these three lines produced an antiviral substance that could, at a 1:2 dilution, suppress the EA expression that follows EBV infection of the interferon sensitive Daudi line by more than 20%. The absence of spontaneous interferon production in the Raji cell line is also in agreement with the results of Zajac *et al.* (1969). In addition to the highly EBV susceptible lines tested here, the RPMI 6410 line, which is only slightly refractory to EBV superinfection (Adams & Klein, 1973), has been reported not to produce any detectable interferon-like activity spontaneously (Zajac *et al.* 1969).
In contrast to the highly EBV susceptible lines, all three of the non-inducible, poorly superinfectable lines clearly produced an antiviral substance (Table 1). These three lines (Namalwa, Akuba and JHTC-33) adsorb EBV normally, but at least a 100-fold higher input multiplicity is required to induce an EA response equivalent to that of the easily superinfectable lines (Adams & Klein, 1973; Nyormoi et al. 1973).

Properties of the antiviral substances produced by the poorly infectable lines

Several tests were performed to determine if the anti-EBV activities spontaneously produced by the various cells had the general properties of interferon (Table 2). The antiviral activities produced by Namalwa, Akuba and JHTC-33 cell lines were inactivated by trypsin digestion but were insensitive to treatment with either DNase or RNase. The active material in these three cell supernatant fluids is apparently a protein. Like interferon, its antiviral activity was non-dialysable but non-sedimentable at 100000g for 2 h. Like leukocyte interferon all samples could be treated for 1 h at 60 °C in medium containing serum without major loss of activity and prolonged exposure to pH 2 did not destroy the antiviral effect investigated here. No difference in anti-EBV titre was found if the Daudi cells were first pre-treated with the various preparations or if the cell supernatant fluids were added after adsorption of EBV. Therefore, the antiviral activity does not act by direct inactivation of the virus or by blocking cell surface EBV receptor sites. Finally, all preparations were similar to human leukocyte interferon in that they suppressed EBV superinfection of Daudi cells while they had little or no effect in preventing EA expression in the Raji cell line. In view of the target cell specificity and the other properties tested in Table 2, it is concluded that the anti-EBV substances produced by Namalwa, Akuba and JHTC-33 cell lines can be classified as an interferon.

The Namalwa cell line has also been found to produce large amounts of interferon upon induction with Sendai virus (Strander, Mogensen & Cantell, 1975) and it was of interest to characterize further the spontaneous interferon of this line. A large batch of Namalwa cells was grown and spontaneous interferon was prepared as described in the Methods section. It was found that the spontaneously produced interferon could be concentrated and purified in the same way as normal human leukocyte interferon (Cantell et al. 1974). The material so purified showed a single small peak with the mobility of beta-globulin on electrophoresis and the activity was abolished by addition of anti-human leukocyte interferon serum. Thus the spontaneously produced Namalwa interferon, like that induced with Sendai virus, is more similar to leukocyte interferon than to human fibroblast interferon, which is poorly neutralized with antisera against human leukocyte interferon (Strander et al. 1975).

The partially purified Namalwa spontaneous interferon had an anti-growth activity as measured on Daudi cells proportional to its antiviral titre as determined by the VSV plaque assay (Adams et al. 1975).

DISCUSSION

The human lymphoid cell lines which are stably transformed with the Epstein–Barr virus (EBV) afford an unique opportunity to study the factors controlling expression of a latent herpes virus in vitro. In a survey of 23 EBV receptor positive lymphoid cell lines, Klein & Dombos (1973) found a significant correlation between a cell's sensitivity to EBV superinfection and its response to activation of intrinsic virus gene functions with iodo- or bromo-deoxyuridine. On the basis of this correlation, they proposed that there was some type of intracellular restriction mechanism, i.e. a negative control, which limited EBV gene expression in the poorly infectable lines.
Employing the Daudi cell line as a model system, it was demonstrated in the previous paper (Adams et al. 1975) that interferon can restrict the expression of EBV early gene functions that follow either virus superinfection or induction with IdU. Interferons, therefore, functions in a fashion analogous to the negative control factor postulated by Klein & Dombos (1973). A further similarity between the proposed intracellular restriction mechanism and interferon is that neither limits the spontaneous antigen expression that occurs in a few per cent of the cells in the so called producer cell lines (Klein & Dombos, 1973; Adams et al. 1975). The concept that interferon may indeed be important in the regulation of EBV gene expression is further supported by the present data. Among 6 EBV receptor positive lymphoid cell lines, selected on the basis of the earlier results to be either extremely susceptible or very refractory to EBV gene expression, only the 3 poorly infectable, non-inducible lines spontaneously produced interferon. The property of 'non-inducibility' in certain EBV-carrying human lymphoid cell lines may therefore not depend on a failure of the thymidine analogues to de-repress the virus, but rather on effective suppression by interferon of the expression of virus lytic functions.

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REFERENCES


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