Sensitivity of the Epstein–Barr Virus Transformed Human Lymphoid Cell Lines to Interferon

By ALICE ADAMS*, H. STRANDER† AND K. CANTELL‡

*Department of Tumor Biology, Karolinska Institute and
†Radiumhemmet, Karolinska Hospital, Stockholm 60, Sweden, and
‡Department of Virology, Central Public Health Laboratory, Helsinki 28, Finland

(Accepted 10 April 1975)

SUMMARY

The effect of interferon on expression of Epstein–Barr virus (EBV) early gene functions was investigated. The ‘early antigen’ synthesis which follows either EBV superinfection of established lymphoid cell lines or 5'-iododeoxyuridine activation of the intrinsic EBV genomes harboured by these cells could be suppressed with interferon. In contrast, the spontaneous early antigen expression that occurs in a few per cent of the cells in the producer cell lines could not be blocked with interferon.

The lymphoid cell lines tested differed in their ability to acquire an antiviral state after exposure to interferon. Several cell lines were also growth inhibited by the interferon preparations. The antiviral and growth inhibitory activities of different interferon preparations could not be separated by a number of criteria.

INTRODUCTION

Interferon has been shown to inhibit both the multiplication and the cellular transformation induced in vitro by many RNA and DNA tumour viruses (for a review see Oxman, 1973). Interferon also appears to have a protective effect on animals infected with oncogenic viruses and to inhibit the growth of some types of tumours (Gresser, 1974). As discussed by Gresser et al. (1967), such results are not unexpected in the case of tumours induced by, e.g. Friend virus, in view of the sensitivity of this RNA virus to the antiviral action of interferon and the apparent involvement of continued virus replication and transformation of previously uninfected cells in the progression of these tumours. In contrast, tumours induced by DNA viruses appear to result from the unrestricted multiplication of a single transformed cell in the absence of virus production. Thus, while pre-treatment with interferon might be anticipated to reduce the tumour incidence in animals infected with interferon-sensitive DNA tumour viruses, treatment with interferon after malignant transformation has occurred may be without effect.

With rare exceptions, human lymphocytes do not grow continuously in culture unless they are transformed by the Epstein–Barr virus; EBV (for reviews see Klein, 1973; Miller, 1974). Human lymphoid cell lines consequently afford an opportunity to test the effect of interferon on a herpes virus-transformed cell under in vitro conditions. We describe here results on the suppressive effect of interferon on the expression of EBV early functions and on the growth of several EBV transformed lymphoid cell lines.
Table 1. Properties of lymphoid cell lines

<table>
<thead>
<tr>
<th>Line</th>
<th>Origin*</th>
<th>Spontaneous virus antigen production†</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akuba</td>
<td>BL</td>
<td>Non-producer</td>
<td>Klein, Dombos &amp; Gothoskar, 1972</td>
</tr>
<tr>
<td>Daudi</td>
<td>BL</td>
<td>Producer</td>
<td>Klein et al. 1967</td>
</tr>
<tr>
<td>DSTC-4</td>
<td>IM</td>
<td>Producer</td>
<td>Junge, Hoekstra &amp; Deinhardt, 1971</td>
</tr>
<tr>
<td>Kamoti</td>
<td>BL</td>
<td>Producer</td>
<td>Nai-Sto†</td>
</tr>
<tr>
<td>LY-46</td>
<td>BL</td>
<td>Producer</td>
<td>Klein et al. 1972</td>
</tr>
<tr>
<td>Maku</td>
<td>BL</td>
<td>Producer</td>
<td>Yata &amp; Klein, 1969</td>
</tr>
<tr>
<td>Naliaka</td>
<td>BL</td>
<td>Producer</td>
<td>Klein et al. 1972</td>
</tr>
<tr>
<td>Namalwa</td>
<td>BL</td>
<td>Non-producer</td>
<td>Klein et al. 1972</td>
</tr>
<tr>
<td>NC-37</td>
<td>N</td>
<td>Producer</td>
<td>Durr et al. 1970</td>
</tr>
<tr>
<td>Odour</td>
<td>BL</td>
<td>Non-producer</td>
<td>Nai-Sto†</td>
</tr>
<tr>
<td>P3HR-1</td>
<td>BL</td>
<td>Producer</td>
<td>Hinuma &amp; Grace, 1967</td>
</tr>
<tr>
<td>Raji</td>
<td>BL</td>
<td>Non-producer</td>
<td>Pulveraft, 1965</td>
</tr>
<tr>
<td>RPMI 6410</td>
<td>L</td>
<td>Non-producer</td>
<td>Ikawata &amp; Grace, 1964</td>
</tr>
<tr>
<td>SKL-1</td>
<td>L</td>
<td>Non-producer</td>
<td>Clarkson, Striss &amp; de Harven, 1967</td>
</tr>
</tbody>
</table>

* BL, Burkitt's lymphoma; IM, infectious mononucleosis; N, derived from normal peripheral blood; L, leukaemia.
† Lines have been classified as producers if > 0.5% early antigen (EA) positive cells are observed in growing cultures.
† A line, recently established in Stockholm, from a Burkitt's lymphoma biopsy taken in Nairobi by Dr S. Singh.

METHODS

Cell lines. The origins of the various human lymphoid cell lines studied are summarized in Table 1. All lines were positive for the EBV-determined nuclear antigen, EBNA, and therefore assumed to be transformed by EBV (Lindahl et al. 1974). The cell lines were grown as suspension cultures in RPMI 1640 medium supplemented with 10% heat-inactivated foetal bovine serum; 100 units/ml penicillin and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO₂. The cultures were maintained by adjusting the cell concentration to 2 × 10⁵ living cells/ml with fresh medium twice weekly. The number of living cells was assessed by the trypan blue exclusion test.

Epstein–Barr virus (EBV). Infectious EBV was concentrated from the spent culture medium of P3HR-1 cells with polyethylene glycol (Adams, 1973). Susceptibility of EBV-transformed lymphoid cells to superinfection with EBV was monitored by determining the number of cells expressing EBV early antigen (EA) two days after infection with EBV (Adams & Klein, 1973; Adams et al. 1975). Acetone fixed cell smears were stained with a 1:40 dilution of the direct FITC-conjugated EA-positive Iketumba reference reagent. Interferon pre-treatment was performed at a cell concentration of 2 to 5 × 10⁵ cells/ml for 16 h at 37 °C. The treated cells were then washed twice with warm medium prior to addition of virus. Induction of EA by treatment of transformed cells with 5-iododeoxyuridine (IdU) was done according to Klein & Dombos (1973).

Vesicular stomatitis virus (VSV). The Indiana strain of VSV was grown in U (human amnion) cells. The preparation used contained 10⁶ p.f.u./ml when titrated in U cells.

Interferon assay by VSV plaque reduction. Threefold serial dilutions of interferon were titrated by the reduction of VSV plaques on U cells (Strander & Cantell, 1966). All units are expressed in terms of the international reference preparation 69/19 (International Symposium on the Standardization of Interferon and Interferon Inducers, London, 1969).

Human leukocyte interferon. The crude interferon was prepared in leukocyte suspensions induced with Sendai virus as described by Cantell et al. (1974). Two types of preparations...
were used in the experiments below: (1) Concentrated interferon (C-IF) was obtained by precipitation of interferon in the presence of 0.5 M-potassium thiocyanate at pH 3.5, dissolution of the sediment in about 1/3 of the volume of 0.1 M-sodium acetate, pH 7.4, dialysis against phosphate buffered saline, and high-speed sedimentation to remove insoluble material (Cantell & Pyhältö, 1973). The C-IF batches contained 1 to 2 × 10⁶ interferon units/ml and had a specific activity (sp. act.) in the order of 20000 to 40000 units/mg protein. (2) Partially purified interferon (P-IF) was obtained by selective precipitation of inert proteins from ethanol solution with rising pH (Cantell et al., 1974). The P-IF batches contained 6 to 20 × 10⁶ interferon units/ml and had a sp. act. of about 10⁸ units/mg protein.

Mock interferon preparations. The leukocyte suspensions were treated in the same fashion as in the routine production of interferon, but normal allantoic fluid was added instead of the Sendai virus. The mock interferon was used (1) as a crude preparation, (2) after about 30-fold concentration like the C-IF preparations or (3) after about 1000-fold concentration and partial purification like the P-IF preparations.

CM-interferon. A P-IF preparation was reduced by mercaptoethanol and carboxymethylated by iodoacetamide as described by Mogensen & Cantell (1974a). This treatment completely destroys the antiviral activity which is dependent upon the integrity of at least one disulphide bond.

Human fibroblast interferon was a gift from Dr Paul Came, Schering Corporation, Bloomfield, N.J. 07003. It contained 6000 units/ml.

RESULTS

Interferon sensitivity of EBV

Herpes viruses as a group have been considered to be relatively insensitive to the antiviral action of interferon in a variety of different cell systems (for a review see Lockart, 1973). As no in vitro lytic system is available for EBV, it is not possible to define the interferon sensitivity of this virus in conventional terms. However, EBV effectively induces an abortive lytic response on infection of some cultured lymphoid cell lines, which can be followed by measuring virus-associated antigen expression with immunofluorescent techniques. In the present study, the effect of interferon on early antigen (EA) synthesis was determined. The early antigen complex is expressed prior to the onset of virus DNA replication and the synthesis of EBV structural proteins (Gergely, Klein & Ernberg, 1971).

Six superinfectable lymphoid cell lines (Raji, Daudi, NC-37, Maku, RPMI 6410 and Odour) were treated with various concentrations of human leukocyte interferon and then infected with EBV. The effect of interferon on the EA expression that follows superinfection of the Raji and Daudi lines is shown in Fig. 1. While untreated cultures of Raji and Daudi cells are equally susceptible to EBV infection (Adams & Klein, 1973), these two lines showed markedly different responses after interferon pre-treatment. With the Daudi line, a 50% inhibition in the number of EA-positive cells was observed with 5 to 10 units/ml of interferon, while with Raji over 500 units/ml were required to reduce the number of antigen positive cells to the same level. The NC-37 line, which is also highly susceptible to EBV superinfection, was similar to Raji in its EA response after exposure to interferon.

The other three lines tested (Maku, RPMI 6410 and Odour) are relatively refractory to superinfection, requiring approx. 20 times more EBV to give an EA response equivalent to that measured with the Raji, Daudi and NC-37 lines (Adams & Klein, 1973). Employing this input multiplicity, the effect of interferon treatment on superinfection of the RPMI 6410 line was found to be similar to that observed with the Raji and NC-37 lines. Maku cells were
slightly more sensitive to interferon, with a 50% reduction in the number of EA-positive cells at 100 units/ml. The Odour cell line, on the other hand, was similar to the Daudi line, with less than 10 units/ml of interferon being sufficient to block EA expression in at least 50% of the infected cells.

Identical results were obtained if, instead of pre-treating the cells with interferon, the interferon was added directly after virus adsorption (Fig. 1). Therefore, even with lines like Raji, which require high doses of interferon to inhibit EA expression, the antiviral effect acts on intracellular virus expression and is not due to the inactivation of extracellular virus or to the blocking of cell surface virus receptor sites.

The same EBV preparation was used to infect all six cell lines tested. The variation in interferon sensitivity observed would therefore seem to be due to differences in the ability of the cells to acquire an antiviral state after interferon treatment. Two cell lines, Raji and Daudi, were also tested for interferon susceptibility with a different virus, unrelated to EBV. The effect of interferon pre-treatment on the growth of VSV in these two cell lines is presented in Table 2. At 48 h after infection, VSV grew as well in Raji cells pre-treated with 1000 units/ml interferon as in the untreated control culture, while with Daudi as little as 10 units/ml of interferon was sufficient to completely inhibit VSV multiplication. Lymphoid cell lines thus differ greatly in the degree that they respond to exogenous human leukocyte interferon, as measured by inhibition of the expression of either an RNA virus or a DNA virus.
Interferon effect on EBV and lymphoid cells

Table 2. Effect of interferon on VSV growth in two human lymphoid cell lines*

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Interferon concentration</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raji</td>
<td>Untreated control</td>
<td>3.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Raji</td>
<td>10 units/ml</td>
<td>3.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Raji</td>
<td>1000 units/ml</td>
<td>2.8</td>
<td>4.0</td>
</tr>
<tr>
<td>Daudi</td>
<td>Untreated control</td>
<td>&lt; 2.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Daudi</td>
<td>10 units/ml</td>
<td>&lt; 2.0</td>
<td>&lt; 2.0</td>
</tr>
<tr>
<td>Daudi</td>
<td>1000 units/ml</td>
<td>&lt; 2.0</td>
<td>&lt; 2.0</td>
</tr>
</tbody>
</table>

* Five x 10^5 lymphoid cells/ml were incubated for 24 h in the presence of various concentrations of interferon (C-IF). The cells were then washed once and VSV added to a multiplicity of 0.06 p.f.u./cell. After 1 h at 37 °C the cells were again washed once and resuspended in fresh medium. Supernatant fluids were harvested at 24 and 48 h post infection and titred for VSV on human amnion cells. Titres are mean values of triplicate samples.

A weak suppressive effect of interferon on VSV growth was observed with the relatively interferon-resistant Raji cells at 24 h (Table 2). Comparing the inhibition of VSV growth in Raji at this time (a 60 % and 75 % reduction in VSV titre with 10 and 1000 interferon units/ml respectively) with that for EBV EA expression (Fig. 1), VSV would appear to be about 100 times more sensitive than EBV to interferon. The observation that VSV multiplication was completely prevented by only 10 units/ml of interferon in the more interferon-sensitive Daudi cell line, while EBV expression was only partly inhibited by this amount of interferon is consistent with such a difference in interferon sensitivity between the two viruses. EBV, like other herpes viruses, therefore seems to be relatively resistant to the antiviral action of interferon.

Effect of interferon on expression of intrinsic EBV genomes

The vast majority of lymphoid cell lines of human origin harbour multiple copies of the EBV genome (zur Hausen et al. 1972; Nonoyama & Pagano, 1973), and all such lines express at least one virus-specific antigen, EBNA (Reedman & Klein, 1973). Additional virus antigens are expressed in some lines after treatment of the cells with halogenated deoxyuridine derivatives (Gerber, 1972; Sugawara, Mizuno & Osato, 1972). Daudi cells can be induced to express EA in 10 to 20 % of the cells by exposure to IdU (Klein & Dombos, 1973). Fig. 2 shows the effect of interferon pre-treatment on the EA expression that follows after IdU induction or EBV superinfection of the Daudi line. It would seem that the EA synthesis that follows either of these two induction processes is equally sensitive to interferon pre-treatment.

With some human lymphoid cell lines, the so-called producer lines, a few per cent of the cells in an actively growing culture spontaneously enter a lytic virus cycle and express various EBV functions, including EA. In contrast to the results on EA expression after either superinfection or IdU induction, it has not been possible to demonstrate an effect of interferon on the spontaneous EA synthesis observed in producer lines such as Daudi, LY-46, and Maku. Because of its marked interferon sensitivity, the Daudi line could only be tested at low interferon concentrations. However, with Maku and LY-46 there was no detectable decrease in spontaneous EA synthesis even after prolonged treatment with relatively high doses of interferon.

One culture of Maku cells grown for 21 days in the continued presence of 10000 interferon units/ml showed no reduction in the number of EA-positive cells over a control culture grown in the absence of interferon (1.3 against 1.2 % EA positive cells were observed). The interferon treated Maku culture was also tested for susceptibility to EBV superinfection at
Fig. 2. Effect of interferon on early antigen expression following IdU induction and EBV superinfection. Cultures of Daudi cells, pre-treated with various concentrations of interferon for 16 h, were divided in two equal portions. One sample was superinfected with EBV while IdU (final concentration of 25 µg/ml) was added to the other portion. The number of EA-positive cells was determined 48 h later. The percentage reduction in antigen-positive cells over that for cells either induced with IdU or superinfected with EBV in the absence of interferon pre-treatment was calculated. ▲, IdU induced culture and ●, EBV superinfected cells.

the end of the 21 day period. The level of EA-positive cells after superinfection with EBV was the same as that observed when an untreated control culture of Maku cells was first superinfected and then exposed to 10000 units/ml interferon immediately after virus adsorption. A 70% against 75% reduction in the number of EA-positive cells was observed, compared to that found for Maku cells superinfected with EBV in the absence of interferon. Therefore, the failure to observe a reduction in the number of cells spontaneously expressing EA was not due to the selection of an interferon resistant sub-population of cells by prolonged growth in the presence of interferon.

**Effect of interferon on the growth of lymphoid cell lines**

EBV is thought to be responsible for the immortalization of human B type lymphocytes as continuously replicating cell lines (for a review see Miller, 1971). The effect of interferon on the growth of 14 such established cell lines was tested. Several of these lines were originally established from Burkitt lymphoma biopsies. The cells were incubated in the continuous presence of interferon, and the number of living cells determined at regular time intervals. Each culture was followed for 4 weeks. The growth responses of four representative lines are shown in Fig. 3.

Six of the 4 lines tested were growth inhibited by low concentrations of the interferon preparation. The inhibitory effect of low interferon concentrations was most apparent after
Interferon effect on EBV and lymphoid cells

Fig. 3. Effect of interferon on the growth of lymphoid cell lines. At weekly intervals the number of living cells in cultures containing various concentrations of interferon was determined and the cultures adjusted to $10^4$ living cells/ml with additional RPMI 1640 medium. Interferon (C-IF) was replenished to the stated concentration and incubation continued at 37 °C. Results are presented as the per cent of living cells in the cultures containing interferon as compared to that of control cultures grown in parallel. (a) Daudi cells grown in the presence of interferon: ○—○, 0.1 units/ml; ▲—▲, 1 unit/ml; ■—■, 10 units/ml, and Raji cells ●—●, with 10000 units/ml of interferon. (b) P3HR-1 cells grown in the presence of interferon: ○—○, 10 units/ml; ▲—▲, 100 units/ml; ■—■, 1000 units/ml and Maku cells, ●—●, grown with 10000 units/ml of interferon.

prolonged incubation periods. The Daudi line (Fig. 3a) was the most sensitive cell line, with 1 interferon unit/ml being sufficient to reduce the total number of living cells by 50% after a 21 day incubation period. The P3HR-1 line required 3 units/ml of interferon in order to similarly reduce cell numbers (Fig. 3b). Of the other lines tested, SKL-1, Naliaka and Odour were similar to the P3HR-1 line in their growth behaviour at various interferon concentrations. The sixth growth inhibitable line, Kamoti, was slightly more resistant, requiring more than 50 units/ml of interferon to reduce the cell number by 50% after 21 days. The inhibitory effect of the interferon preparation in these experiments was not of an immediately toxic nature. Hence, even with Daudi, the most sensitive of the six lines, an effect on the cell number was not apparent until the third or fourth day after addition of interferon. Moreover, the block in cell multiplication could be reversed by washing the cells free of interferon. In one experiment, Daudi cells were exposed to 100 units/ml interferon for four days and then washed and incubated in the absence of interferon. Growth of this culture resumed after 2 days and the rate of cell division was normal after 4 days. Samples of the culture were also tested for susceptibility to EBV superinfection. After 2 days in the
absence of interferon, the initial antiviral state had decayed by 50% and on the fourth day, when growth was normal the cells were fully susceptible to EBV.

In marked contrast to the six growth inhibitable lines, the other eight lines tested grew normally in the presence of high concentrations of interferon. In addition to the Raji and Maku lines (Fig. 3), the lines LY-46, NC-37, Namalwa, DSTC-4, RPMI 6410 and Akuba were not measurably growth inhibited after 28 days in the continued presence of $10^4$ interferon units/ml. The Raji, Maku, NC-37 and LY-46 lines were also tested and found to be resistant to $10^5$ interferon units/ml.

Among those lines which are sufficiently susceptible to exogenous EBV to test for an interferon effect on virus superinfection, a good correlation exists between the antiviral and growth responses of the line. Thus, EBV-induced EA synthesis was easily suppressed after interferon treatment of the growth inhibitable Daudi and Odour lines, while high interferon doses were required to effect the superinfection of the NC-37, Raji, Maku and RPMI 6410 lines. In addition, an antiviral effect of interferon on the spontaneous EBV production of the growth inhibitable P3HR-I line has been demonstrated (A. Adams, unpublished data). This correlation suggests that interferon itself, and not some other substance in the interferon preparation, is the growth inhibitory substance.

Evidence for interferon nature of the growth inhibitor

Several additional experiments were performed in an attempt to substantiate whether the growth inhibitory substance was interferon. Interferon preparations of different purities and origins were analysed for their relative antiviral and growth inhibitory activities. Both concentrated (C-IF) and the 50-fold purified (P-IF) human leukocyte interferons were found to have growth inhibitory activities proportional to their interferon titres. Human fibroblast interferon and the antiviral activity spontaneously produced by the Namalwa cell line (Adams et al. 1975) likewise had the same relative growth inhibitory to antiviral activities.

Three mock interferon preparations and the inactivated CM-interferon were all without effect on the growth of the Daudi cell line, even when tested at concentrations which would be equivalent to $10^5$ "interferon" units/ml. Human leukocyte interferon at concentrations up to $10^4$ units/ml had no effect on the growth of two mouse cell lines (YCAB-1, a lymphoma derived line, and Ehrlich ascites carcinoma cells).

The growth inhibitory activity was non-dialysable and non-sedimentable (2 h at 100,000 g). The activity was destroyed by trypsin digestion and the inhibitor, like interferon, would appear to be a protein. The inhibitor precipitated with interferon when crude leukocyte interferon preparations were heated at 70 °C for 1 h and both activities could be recovered from the pellet by treatment with 4 M-guanidine-HCl (Mogensen & Cantell, 1973). Moreover, when 50-fold purified leukocyte interferon (P-IF) was heated in phosphate buffered saline, the antiviral and growth inhibitory activities were found to exhibit the same kinetics of inactivation both at 60 and at 75 °C. A sheep antiserum prepared against purified human leukocyte interferon (P-IF) neutralized the growth inhibitory activity of 50 units of interferon at a dilution of 1:20,000. The neutralization titre of this antiserum against human leukocyte interferon in the VSV plaque reduction test was 1:150,000 (Mogensen & Cantell, 1974b).

DISCUSSION

Following a primary infection, the Epstein–Barr virus is carried by B type lymphocytes in a latent form for long periods of time, possibly for the life of the individual. The factors limiting the expression of such latent herpes viruses remain poorly understood. Here the
expression of EBV-specified early functions have been demonstrated to be suppressible with interferon, and while EBV does not seem to be as sensitive to interferon treatment as VSV, as little as 10 units/ml of interferon was still sufficient to effectively block EA expression in some of the lymphoid cell lines.

Many of the established lymphoid cell lines were found to be extremely insensitive to treatment with exogenous interferon. Since several lymphoid cell lines are known to spontaneously produce interferon, it is feasible that the relative insensitivity of some of the cell lines to interferon treatment is a result of *in vitro* cultivation. Selection of interferon resistant cells seems quite likely in view of the finding that interferon was growth inhibitory to the class of cells in which an antiviral state could be most easily demonstrated. These latter, interferon sensitive lines were presumably established in culture in the absence of significant levels of interferon.

The abortive lytic virus cycle which follows either EBV superinfection or activation of intrinsic virus genomes with IdU is sensitive to the action of interferon. In contrast, the spontaneous antigen expression in the producer cell lines studied here could not be suppressed with interferon. These observations indicate that the mechanisms of IdU and spontaneously induced EA synthesis may be different. One possibility is that different types of EBV genomes are activated: both free, episome-like virus genomes and EBV DNA covalently integrated into the cellular DNA are present in the Raji cell line (Adams & Lindahl, 1975). Alternatively, the failure of interferon to suppress spontaneous antigen synthesis could be due to an increased resistance to interferon of those cells where induction of the virus cycle occurs. The proportion of cells spontaneously expressing early antigen increases as the cell cultures go into stationary phase. It is therefore possible that only metabolically defective cells spontaneously express EBV-related antigens, and such cells may be unable to attain an antiviral state induced by interferon.

A growth inhibitory effect of various interferon preparations on many established cell lines of widely different origin has been observed by several investigators (for a review see Gresser, 1972). In the present study the growth inhibitory activity of human interferon preparations on human lymphoid cell lines could not be separated from the antiviral activity by a number of criteria. Final proof that the anti-growth substance is interferon itself will require access to interferon purified to homogeneity. Assuming interferon to be the growth inhibitory substance there remains the question of the mechanism of action. EBV is thought to be responsible for transforming or immortalizing human B type lymphocytes into continuously replicating cell lines, and the growth inhibition observed here could be due to interferon suppression of a continuously expressed virus gene function, essential to cellular growth *in vitro*. Further studies directed at determining the effect of interferon on EBV gene functions which, in contrast to EA, are continually expressed in these cells will be needed to elucidate this problem.

We thank Dr K. E. Mogensen for the preparation of CM interferon. The technical assistance of Ms Ann Christine Synnerholm and Ms Waltraut Szczesna is gratefully acknowledged. The work was supported by grants to A.A. from the Swedish Cancer Society and to H.S. from the Cancer Society of Stockholm. The research was further supported by a contract to G. Klein (No. 1 CP 33316) within the Virus Cancer Program of the U.S. National Cancer Institute.
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Interferon effect on EBV and lymphoid cells


(Received 21 January 1975)