Interferon Induction and Action in Human Lymphoblastoid Cells Infected with Measles Virus

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SUMMARY

Virus replication, cytopathic effect and interferon production were measured in Namalva lymphoblastoid cells infected with measles viruses. Eight virus strains of different origin or passage history were compared. An inverse relationship seemed to exist between the abilities of strains to induce interferon and to replicate to high titres. Two representative strains were found to be highly sensitive to lymphoblastoid cell interferon, when tested in a line of monkey kidney cells (Vero). In contrast, Namalva cells were found to be highly insensitive to autologous interferon when challenged with measles or Semliki Forest virus (SFV).

INTRODUCTION

Measles virus replicates and induces interferon in Namalva lymphoblastoid cells (Volckaert-Vervliet & Billiau, 1977; De Ley et al. 1978). Analysis of the virus growth curve revealed that considerable increments in virus titre occurred at a time when high titres of interferon were already present in the culture supernatant. Seemingly, the endogenous interferon had little or no effect on the replication of the virus. The present study was initiated to analyse this phenomenon.

METHODS

Most of the materials and methods used in the present study were described in a previous paper (Volckaert-Vervliet & Billiau, 1977). Interferon titres are expressed in terms of the M.R.C. reference preparation 69/19. Lymphoblastoid cell interferon was prepared by infection of Namalva cells with measles virus as described elsewhere (De Ley et al. 1978). It was used as a crude preparation containing $10^{4}$ units/ml in medium with 10% foetal calf serum. The following strains of measles virus were used: A, a commercially available vaccine strain (Attenuvax) kindly provided by the manufacturer (Merck, Sharp and Dohme, Haarlem, The Netherlands); RO, a commercially available vaccine strain (Rouvax, Mérieux, Lyon, France); M18, a Schwartz strain obtained from Dr N. Zygraich (R.I.T., Genval, Belgium); F9, an Edmonston strain, obtained from Dr E. A. Gould (The Queen’s University, Belfast, N. Ireland); V, a strain obtained from Dr J. Melnick (Baylor University, Houston, Texas, where it was considered to be a non-attenuated Edmonston–Enders strain); LU, an Edmonston-B strain obtained from Dr C. J. Lucas (Central Laboratory,
Table I. Passage of different measles virus strains in Namalva lymphoblastoid cells: virus replication, cytopathic effect and interferon induction*

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Average duration (days)</th>
<th>Number</th>
<th>Virus yield (log_10 TCID_{50}/ml)</th>
<th>Viable cells at harvest (%)</th>
<th>Interferon yield (log_10 units/10^6 cells added)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.6</td>
<td>7</td>
<td>5.34 (0.80)†</td>
<td>45.0 (5.2)†</td>
<td>3.30 (0.16)†</td>
</tr>
<tr>
<td>RO</td>
<td>3.6</td>
<td>5</td>
<td>6.06 (0.35)</td>
<td>35.0 (8.5)</td>
<td>2.89 (0.40)</td>
</tr>
<tr>
<td>M18</td>
<td>3.6</td>
<td>5</td>
<td>5.77 (0.48)</td>
<td>35.8 (16.0)</td>
<td>2.88 (0.16)</td>
</tr>
<tr>
<td>RI</td>
<td>5.2</td>
<td>4</td>
<td>5.80 (0.62)</td>
<td>45.5 (6.8)</td>
<td>2.70 (0.28)</td>
</tr>
<tr>
<td>P9</td>
<td>8.3</td>
<td>3</td>
<td>5.60 (0.31)</td>
<td>57.0 (13.4)</td>
<td>2.43 (0.26)</td>
</tr>
<tr>
<td>V</td>
<td>5.5</td>
<td>2</td>
<td>6.27 (0.46)</td>
<td>50.7 (2.8)</td>
<td>2.33 (0.05)</td>
</tr>
<tr>
<td>LU</td>
<td>3.0</td>
<td>6</td>
<td>&gt; 6.50</td>
<td>39.2 (5.1)</td>
<td>1.60 (0.45)</td>
</tr>
<tr>
<td>LT</td>
<td>2.0</td>
<td>7</td>
<td>&gt; 6.80</td>
<td>11.5 (5.9)</td>
<td>&lt; 1.00</td>
</tr>
</tbody>
</table>

* Passage by adding fresh cells to sedimented infected cells from previous passage (see text).
† Mean values excluding figures from first harvest; standard deviation in parentheses.

Blood Transfusion Service, Amsterdam, The Netherlands); and LT, a wild-type strain obtained from Dr L. Thiry (Institut Pasteur du Brabant, Brussels, Belgium). Semliki Forest virus (SFV) was originally obtained from Dr R. Friedman (National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland); it was propagated on Vero or Namalva cells and titrated by plaque formation on Vero cells.

RESULTS

Three approaches were used to analyse the apparent insensitivity to interferon of the Namalva cell/measles virus system. (1) Several strains of measles virus were examined for virus replication and interferon induction to see whether these two parameters are inversely related. (2) The interferon sensitivity of two representative strains of measles virus was determined in Vero cells. (3) The interferon sensitivity of Namalva lymphoblasts infected with different challenge viruses was studied.

Comparative study of virus replication and interferon production in Namalva cells infected with different strains of measles virus

Namalva lymphoblastoid cell suspensions (25 ml at 2 × 10^6 cells/ml) were inoculated with different measles virus strains. Twice weekly the cells were counted, the supernatant fluids were harvested and the cultures were re-fed with fresh medium. As soon as the cell viability dropped below 50%, the sedimented cells were added to fresh suspensions of uninfected Namalva cells (25 ml at 2 × 10^6 cells/ml). These passages were repeated several times for each virus. Table I shows the average time intervals necessary for each virus strain to cause a drop of 50% or more in cell viability. It also shows the yields of virus and of interferons in the supernatant fluids. Considerable variation in cytopathogenicity, growth rate and interferon-inducing potential can be seen. Most of the available strains were attenuated to a certain extent and induced rather high amounts of interferon. The A strain invariably gave the best yields. This was also seen in other experiments not shown in this paper. Two strains (LT and LU) induced low or undetectable levels of interferon. The same strains also replicated to higher titres, and the LT strain caused very rapid c.p.e. Thus low interferon levels seemed to correlate with rapid virus growth. From this, one could infer
Interferon and measles in lymphoblastoid cells

Fig. 1. Inhibition of the replication of measles virus in Vero cells, pre-treated for 24 h with different concentrations of lymphoblastoid cell interferon. ○—○, LT strain, m.o.i. = 100 TCID\text{50}/cell, 2 day yield; ●—●, LT strain, m.o.i. = 0.1 TCID\text{50}/cell, 5 day yield; △—△, A strain, m.o.i. = 1 TCID\text{50}/cell, 3 day yield; ▲—▲, A strain, m.o.i. = 0.03 TCID\text{50}/cell, 8 day yield.

that endogenous interferon is a regulatory factor for the replicative and cytolytic functions of measles virus in Namalva cells.

Sensitivity of measles virus to lymphoblastoid cell interferon in Vero cells

We verified that the LT strain of measles virus as well as the A strain, a representative vaccine strain, were inherently sensitive to exogenous lymphoblastoid cell interferon. We chose Vero cells as an indicator cell line because they are sensitive to the antiviral effect of interferon and do not produce interferon. The cell monolayers were incubated for 24 h with different concentrations of interferon, washed once and inoculated with measles virus at different multiplicities of infection (m.o.i.). After a 90 min adsorption period, excess virus was removed by three washes. The cultures were re-fed with fresh medium and incubated till c.p.e. became apparent in the control cultures which were not treated with interferon. At various times, samples of the supernatant fluid were taken and assayed for their infectious virus content. The effect of lymphoblastoid cell interferon on both strains of measles virus is shown in Fig. 1 in the form of dose-dependent yield reduction curves. From these experiments it is clear that both strains were highly sensitive to lymphoblastoid cell interferon when tested on Vero cells.
Fig. 2. Effect of lymphoblastoid cell interferon on cell growth and on the cytopathic effect of VSV in Namalva cells. Interferon was present throughout the experiment at 0 units/ml (○—○), 300 units/ml (■—■), 1000 units/ml (▲—▲) or 3000 units/ml (●—●). (a) VSV was inoculated at a m.o.i. of 2.5 p.f.u./cell; (b) uninfected control cultures.

Table 2. Virus titres in Namalva cell cultures treated with different concentrations of interferon and inoculated with VSV at a m.o.i. of 2.5 p.f.u./cell

<table>
<thead>
<tr>
<th>Concentration of interferon (units/ml)</th>
<th>Virus titre (log p.f.u./ml) after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td>0</td>
<td>5.7</td>
</tr>
<tr>
<td>300</td>
<td>4.6</td>
</tr>
<tr>
<td>1000</td>
<td>4.4</td>
</tr>
<tr>
<td>3000</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* The same experiment as shown in Fig. 2.

Sensitivity of Namalva cells to the antiviral effect of lymphoblastoid cell interferon

Comparative measurements of the sensitivity of cell lines to the antiviral effect of interferon require the use of single cycle yield reduction assays. The challenge virus should fulfil the following minimum requirements: it must (1) have a known susceptibility to interferon in other (indicator) cell lines; (2) replicate quickly to avoid leakage due to waning of the interferon effect; (3) replicate to a titre sufficiently high to be distinguishable from the titre of residual input virus and (4) not induce the production of interferon during the assay. Vesicular stomatitis virus (VSV) fulfils these conditions in most cell systems ordinarily used in interferon research. However we found it unsuitable for testing the sensitivity of Namalva lymphoblastoid cells. The following representative experiment illustrates this. Two sets of Namalva cell cultures (10^6 cells/ml, 10 ml) were treated overnight with different concentrations of lymphoblastoid cell interferon. One set of the suspensions was inoculated with VSV at a m.o.i. of 2.5 p.f.u./cell and incubated for 2 h. Both sets were then washed three times and the cells were resuspended in fresh medium and adjusted to a concentration of 10^6 cells/ml in all cultures. Interferon was added at the desired final concentration and the cultures were incubated for 6 days. At daily intervals the cells were counted. Also, samples
Table 3. Effect of lymphoblastoid cell interferon on measles virus (LT strain) in Namalva cells*

<table>
<thead>
<tr>
<th>Concentrations of interferon (units/ml)</th>
<th>Increase (%) in the number of viable cells from 2 to 24 h</th>
<th>Percent viable cells at 24 h</th>
<th>Virus yield (log TCID$_{50}$/ml) at 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected</td>
<td>Infected</td>
<td>Uninfected</td>
</tr>
<tr>
<td>0</td>
<td>9</td>
<td>-23</td>
<td>92</td>
</tr>
<tr>
<td>300</td>
<td>6</td>
<td>-20</td>
<td>89</td>
</tr>
<tr>
<td>1000</td>
<td>11</td>
<td>-23</td>
<td>87</td>
</tr>
<tr>
<td>3000</td>
<td>-6</td>
<td>-7</td>
<td>85</td>
</tr>
</tbody>
</table>

* Cell suspensions (1 × 10$^6$ cells/ml) were incubated for 24 h with interferon, inoculated with virus (m.o.i. = 1), then washed and re-incubated with fresh, interferon-free medium for 24 h. Cell numbers and virus yields were determined on samples taken at 2 and 24 h after removal of interferon.

Table 4. Cytopathic effect and replication of SFV in control and interferon-treated Namalva cells*

<table>
<thead>
<tr>
<th>Concentration of interferon (units/ml)</th>
<th>Viable cells (× 10$^{-4}$/ml) at different times after infection</th>
<th>Virus yields (log$_{10}$ p.f.u./ml) at different times after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>24 h</td>
</tr>
<tr>
<td>0</td>
<td>1.7 (94)†</td>
<td>1.4 (73)†</td>
</tr>
<tr>
<td>30</td>
<td>1.4 (88)</td>
<td>1.3 (66)</td>
</tr>
<tr>
<td>100</td>
<td>1.3 (95)</td>
<td>1.3 (65)</td>
</tr>
<tr>
<td>300</td>
<td>1.4 (93)</td>
<td>1.0 (63)</td>
</tr>
<tr>
<td>1000</td>
<td>1.8 (93)</td>
<td>1.0 (60)</td>
</tr>
<tr>
<td>3000</td>
<td>1.4 (88)</td>
<td>0.8 (52)</td>
</tr>
</tbody>
</table>

* Suspensions of 1.5 × 10$^6$ cells/ml were treated for 24 h with interferon and challenged at time -2 h with SFV at a m.o.i. of 10. Values are means from duplicate cultures.

† % viable cells in parentheses.
cell line (Vero), and failure to induce endogenous interferon. Suspensions of Namalva cells
(10^6 cells/ml, 10 ml) were incubated for 24 h with different concentrations of lymphoblastoid
cell interferon. The cells were washed once, re-fed with fresh medium and adjusted to
10^6 cells/ml. One set of cultures was inoculated with LT measles virus at an added m.o.i.
of 1 TCID_{50}/cell. The other set served as a control. After 2 h incubation all the cultures
were washed three times, re-fed with fresh medium and re-incubated. Samples of the whole
cell suspension were taken for virus titration immediately after washing and at 24 h. The
results are shown in Table 3. In uninfected cultures the cells continued to grow after ex-
posure to interferon at concentrations of 0, 300 or 1000 units/ml, but not at 3000 units/ml.
Infection with measles virus resulted in cell destruction, as indicated by lower numbers of
viable cells at 24 h than at 2 h post-infection. The percentage of viable cells was also
decreased by infection and a slight protection against this c.p.e. of measles virus was
observed with a high concentration of interferon (3000 units/ml). Fast and profuse replica-
tion of the LT measles virus is apparent from the large increase in titres during the 24 h
incubation period and from the large burst size (100 TCID_{50} per cell). Under these condi-
tions interferon at a concentration as high as 3000 units/ml had no effect on the virus
yield. In other similar experiments a marginal yield reduction (0.5 log_{10}) was sometimes seen
with \geq 1000 units/ml. From these results it was concluded that the sensitivity of the
Namalva cell/measles virus system to the antiviral effect of lymphoblastoid cell interferon
is extremely low or non-existent.

The insensitivity of measles virus to exogenous interferon when tested in Namalva cells
was confirmed in experiments employing leukocyte interferon at concentrations up to
20000 units/ml (results not shown) and fibroblast interferon at concentrations up to
1000 units/ml (V. G. Edy, personal communication).

It is possible, but unlikely, that Namalva cells are responsive to interferon, but that
measles virus, although interferon-sensitive in Vero cells, is insensitive in Namalva cells.
To rule out this possibility we decided to use yet another challenge virus. Hilfenhaus (1976)
recommended SFV because it causes c.p.e. and replicates well in lymphoblastoid cell lines.
Table 4 shows the results of an experiment in which suspensions of Namalva cells
(1.5 \times 10^6 cells/ml) were incubated for 24 h with increasing concentrations of lympho-
blastoid cell interferon and were subsequently challenged with SFV at a m.o.i. of 10. After
virus adsorption (2 h at 37 °C) the cells were washed twice and re-fed with plain medium.
Cell counts and infectious virus assays were done on samples of the cell suspensions taken
at the indicated times. It can be seen that interferon did not prevent cell death. Virus
replication was marginally inhibited (0.5 log_{10} difference compared with the control cul-
tures not treated with interferon) by small as well as large concentrations of interferon.
Possibly Namalva cells can respond to fairly small concentrations of interferon, but are
limited in the extent to which they can mount an antiviral state.

**DISCUSSION**

The experiments described were done to analyse the observation that some strains of
measles virus replicate in lymphoblastoid cells despite the presence of relatively high
concentrations of endogenous interferon. It is known that strains (De Maeyer & Enders,
1965) or mutants (McKimm & Rapp, 1977) of measles virus differ greatly in their ability
to induce interferon in various cell systems. If the lymphoblastoid cell/measles virus system
is sensitive to autologous interferon, one would expect that strains which induce interferon
would be auto-inhibited and that non-inducing strains would replicate unrestrictedly. A
comparative study of interferon induction and replication of eight strains of measles virus in Namalva cells seems to confirm this prediction. In further experiments the interferon-sensitivity of measles virus and of Namalva cells were examined separately. A wild type (LT) as well as a vaccine strain (A) of measles virus were both found to be highly sensitive to lymphoblastoid cell interferon when tested on Vero cells. The LT strain replicated easily in Namalva cells; it attained high titres and did not induce measurable interferon. Therefore it was considered a suitable challenge virus to compare the sensitivity of Namalva lymphoblastoid cells to that of an indicator cell line (Vero cells). It was found that concentrations of lymphoblast interferon as high as 3000 units/ml did not significantly inhibit the single-cycle growth rate of LT virus. These data clearly show that Namalva cells have a severely limited ability to develop an antiviral state after exposure to interferon. Experiments employing SFV as a challenge virus led to the same conclusion. Although interferon did not inhibit the cytopathic effect of SFV, marginal decreases in virus yield (0.5 log_{10}) were noted with small as well as large doses of interferon. This suggests that Namalva cells might be limited not so much in their ability to respond to small doses of interferon, but rather in their ability to mount an efficient antiviral state. Perhaps they can only synthesize limited amounts of the hypothetical antiviral protein. This might explain why strains of measles virus which induce interferon have a slower growth curve than non-inducing strains, but continue to replicate after high concentrations of interferon have been released into the medium.

Our findings seem to be in disagreement with those of other authors who found that Namalva cells were rather sensitive to leukocyte interferon (Lidin & Adams, 1975). In these studies the antiviral effect was measured by the inhibition of VSV or Epstein–Barr virus. However, the replication of both these viruses in normal Namalva cells seems to be restricted by unknown factors, as has been described in some but not all lymphoblastoid cell lines (Nowakowski et al. 1973). This inherent restriction might act synergistically with added interferon. Therefore we suggest that measurements made with these viruses have led to an overestimation of the antiviral effect of interferon in lymphoblastoid cells. However, there may be other explanations for the discrepancy between our results and those reported by others.

The apparent low sensitivity of Namalva lymphoblastoid cells to the antiviral effect of interferon raises questions concerning their sensitivity to the growth inhibitory effect of interferon. In our studies the multiplication of Namalva cells was only inhibited if 1000 to 3000 units/ml of interferon was applied. Much higher sensitivities have been reported for other lymphoblastoid cell lines (Adams et al. 1975; Einhorn & Strander, 1977). We cannot ascertain that the growth inhibitory effect seen in our study is specific, since the interferon preparations were not purified. Moreover cell counts were used to evaluate the c.p.e. of viruses; more sensitive systems, such as those used by Gresser et al. (1970) or Einhorn & Strander (1977), would be needed to evaluate accurately inhibition of cell growth. Finally, a general statement concerning the correlation of antiviral and growth inhibitory effects of interferon in human lymphoblastoid cells would require a comparative study on a large number of cell lines or clones as has been done for mouse L-1210 cells (Gresser et al. 1974).

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REFERENCES


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