Recombinant Human Interferon-γ Inhibits Adenovirus Multiplication in vitro

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(Accepted 21 November 1986)

SUMMARY

The susceptibility of adenovirus to the inhibitory effect of human interferons in vitro was investigated. We tested recombinant human interferons-α2, -β1 and -γ against adenovirus serotypes 1 and 5 (group C), 3 and 7a (group B), and a wild strain isolated from an acutely ill child who later died. Pretreatment of WISH cells with interferon-γ for 24 h induced a dose-dependent inhibition of multiplication of all adenovirus strains tested, the TCID<sub>50</sub> varying from 25 to 90 IU/ml. Human interferon-α2 was unable to decrease adenovirus multiplication, while interferon-β at 2000 IU/ml slightly lowered the yield of adenovirus. Similar results were obtained in HEp-2 and FS-4 cells, while A-549 and peripheral blood mononuclear cells were insensitive to interferon-γ. The difference between the effects of interferon-γ and interferon-α and -β on adenovirus multiplication in vitro suggests that its mechanism of antiviral action is different.

Adenoviruses are amongst the most frequent causal agents of acute respiratory illness in childhood as well as of several other important diseases (Schmitz et al., 1983). Some infections can result in the persistence of the virus in intestines or tonsils for months or even years (Fox et al., 1969; Andiman & Miller, 1982). At present, no effective systemic treatment against these viruses is available (De Clercq, 1985).

Although interferons induce an effective antiviral state against different viruses, many adenovirus strains have been found to be relatively resistant to the action of interferon in vitro (for review, see Stewart, 1979). However, the effects of interferon upon adenovirus have been studied mainly with leukocyte interferons, prior to the availability of interferon produced by DNA recombination techniques. The aim of this study was to determine the antiviral activity in vitro of recombinant human interferons-α2, -β1 and -γ against some of the most frequently isolated adenoviruses. Our results showed that interferon-γ was the only type able to inhibit adenovirus multiplication in WISH, HEp-2 and FS-4 cells, and that lymphocytes and A-549 cells were insensitive to its antiviral action against adenovirus.

The adenoviruses used were serotypes 1 (NIH strain), 3 (GB strain, ATCC), 5 (kindly provided by Dr M. Perricaudet, Villejuif, France) and 7a (S-1058 strain, ATCC) and a clinical isolate (A.A.) from the respiratory secretion of a 6-month-old child who later died. The isolation was performed by A.S.M. in the ‘Ricardo Gutiérrez’ Children’s Hospital, Buenos Aires, Argentina; by serotyping and restriction endonuclease pattern it was identified as adenovirus type 3. These strains correspond to groups B (1 and 5) and C (3 and 7a).

Human amniotic WISH cells [3 × 10<sup>5</sup> cells/ml in Eagle’s MEM supplemented with 10% foetal calf serum (FSC)] were seeded, at 0.5 ml/well, into 24-well flat-bottomed tissue culture plates (Nunc) and incubated for 24 h at 37 °C in 5% CO<sub>2</sub> humidified atmosphere. The medium was then discarded and replaced for 24 h by a medium containing the human interferons-α2 (Schering), -β1 (Cetus Corp, U.S.A.) or -γ (Roussel-Uclaf, France) at different concentrations.

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All interferons were pure recombinant DNA products formed in *Escherichia coli*. Thereafter, the medium was removed and the cells were challenged with adenovirus (1 p.f.u./cell) for 60 min at 37 °C. Unadsorbed virus was removed by washing three times with phosphate-buffered saline. After 28 h incubation in Eagle's MEM with 5% FCS at 37 °C, the cells were disrupted by freeze–thawing and the virus titres expressed as reciprocals of dilutions causing 50% cytopathic effect (TCID$_{50}$) in WISH cells. The same protocol was used for human peripheral blood mononuclear cells (PBMC), obtained from normal donors at the Haemotherapy Service of Hôtel-Dieu, Paris.

For all adenovirus strains tested, *in vitro* multiplication was inhibited in a dose-dependent fashion by interferon-γ pretreatment of WISH cells, as shown in Fig. 1. Although the virus yields in WISH cell monolayers were different for each strain, pretreatment with interferon-γ resulted in strong inhibition of multiplication for all the adenovirus strains tested, even the clinical isolate. This inhibition was of at least 2 log$_{10}$ units for adenovirus types 1 and 7a, 3 log$_{10}$ units for adenovirus type 3 and A.A., and 5 log$_{10}$ units for adenovirus type 5. However, they were not equally susceptible to the action of interferon. Thirty IU/ml interferon-γ inhibited adenovirus type 5 and A.A. multiplication by 50% while 90 IU/ml was needed to reach the same level of inhibition for adenovirus type 7a. These results are in agreement with those presented in Table 1 for WISH cells. The phenotypic differences in susceptibility between adenovirus strains were reproducible findings in a set of four experiments.

WISH cells are very sensitive to the antiviral action of interferons-α and -β against many viruses, including vesicular stomatitis virus (VSV) and encephalomyocarditis virus. However, interferon-α, had no activity against any of the adenoviruses tested, even at a concentration as high as 20000 IU/ml (data not shown). The resistance of adenovirus to the action of recombinant interferon-α is in accordance with previous results obtained with leukocyte interferon (Stewart, 1979). It has been suggested recently that adenovirus resistance to interferon-α is related to the accumulation of a viral RNA which prevents the activation of interferon-induced eIF-2 kinase (Kitajewski *et al.*, 1986).

Recombinant interferon-β, also lacked antiviral activity against adenovirus *in vitro*. Romano *et al.* (1980) have reported a therapeutic effect of natural interferon-β, on epidemic keratoconjunctivitis, caused by adenovirus type 8. However, in our hands, recombinant
Table 1. Interferon-γ titres (IU/ml) obtained in different cell types against adenoviruses*

<table>
<thead>
<tr>
<th>Cell</th>
<th>Type</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7a</th>
</tr>
</thead>
<tbody>
<tr>
<td>WISH</td>
<td></td>
<td>4200</td>
<td>2500</td>
<td>10000</td>
<td>625</td>
</tr>
<tr>
<td>HEp-2</td>
<td></td>
<td>625</td>
<td>625</td>
<td>1875</td>
<td>625</td>
</tr>
<tr>
<td>FS-4</td>
<td></td>
<td>3125</td>
<td>6250</td>
<td>4700</td>
<td>3125</td>
</tr>
<tr>
<td>A-549</td>
<td></td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

* The titre of the interferon-γ preparation against VSV in WISH cells was 10000 IU/ml.

interferon-β1 at a concentration of ≥ 2000 IU/ml only decreased adenovirus recovery from the cultures by a factor of 10 (data not shown).

To see whether the inhibition of adenovirus multiplication was limited to WISH cells, we performed additional experiments on other cell types. PBMC were completely insensitive to the anti-adenovirus action of interferon-γ in concentrations up to 250 IU/ml (data not shown). As in the WISH cell system, interferons-α2 and -β1 were also ineffective. The lack of activity of human interferon-γ on PBMC is consistent with the previous results of Orchansky et al. (1986) on monocytes, as well as with our own results against VSV in lymphocytes (M. C. Saavedra & R. Falcoff, unpublished).

The action of interferon-γ against adenovirus on HEp-2 (larynx carcinoma), A-549 (lung adenocarcinoma) and FS-4 (normal fibroblast cells) was tested with a simplified protocol, adapted from Rubin & Gupta (1980). In brief, the same interferon preparation (10000 IU/ml) was titrated on each of these cells, the different adenovirus being the challenge. After 28 h the cultures were evaluated by microscopic observation and the titre expressed as the reciprocal of the dilution which produced 50% inhibition of the cytopathic effect (Table 1). HEp-2 and FS-4 cells behaved similarly to WISH cells, being insensitive to the anti-adenovirus action of interferon-γ against all adenovirus strains tested. However, titres obtained in HEp-2 cells were systematically lower than in WISH cells, suggesting that HEp-2 cells are less sensitive to interferon-γ antiviral action. Titres in FS-4 did not show any particular pattern when compared to WISH cells. A-549 cells were virtually deprived of any adenovirus resistance induced by interferon-γ; VSV was also poorly inhibited in this culture by interferon-γ (not shown). Interferons-α2 and -β1 were unable to inhibit adenovirus cytopathic effect in all cell lines; however, they did inhibit VSV multiplication (data not shown).

In conclusion, amongst the three recombinant human interferons compared, interferon-γ is the only one able to inhibit, dose-dependently, the multiplication of several strains of adenovirus in different cell types. Further studies are currently underway to determine the molecular basis of the anti-adenovirus action of interferon-γ. Taking into account the results presented here and the severity of many adenovirus infections, therapeutic trials with interferon-γ might be justified.

We are indebted to Dr Roberto A. Diez for assistance and suggestions in the planning of this work, to Drs Jeanne Wietzerbin and Saül Grinstein for their encouragement, to Ms Corinne Gaudelet for the supply of WISH cells and to Ms Agnes Gehant for skillful typing of the manuscript.

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(Received 26 August 1986)