HVJ (Sendai Virus) Stimulates Release of Interferon from Leukocytes Used Once for Interferon Production

By KAYOKO MATSUMOTO,* YASUKO NAKAMURA, KATSUHIKO AKASHI AND HIDEO YAMAGUCHI
The Japanese Red Cross, Osaka Blood Center, Morinomiya, Joto-ku, Osaka 536, Japan

(Accepted 21 January 1986)

SUMMARY

Leukocytes, subjected once to interferon (IFN) induction by HVJ (Sendai virus), were studied for their capability to produce IFN after a second similar stimulus. Substantial amounts of IFN (about 30000 IU/ml) were recovered. Experiments using cycloheximide or actinomycin D and kinetic studies showed that this IFN originated mainly in IFN which resided within the cell as a result of the first induction and was released after the second stimulation. Increasing amounts of HVJ used for the second stimulus resulted in proportionally increased yields of IFN, reaching a plateau at the same dose of HVJ (1000 HAU/ml) as that which gave optimal yields after the first stimulation. Evidence is presented that the capacity of HVJ to trigger the production of a second IFN harvest is closely associated with its infectivity.

Interferon (IFN)-α derived from leukocytes of healthy volunteers is produced at the Osaka Blood Center using the method of Mogensen & Cantell (1977). Despite the advantages of this system, leukocytes are only available in limited quantity. Therefore, we investigated the possibility of re-use of the leukocytes for IFN production, and found that a second yield of about 30000 International Units (IU)/ml of IFN could be obtained.

The pooled buffy coat layers from healthy donors were centrifuged at 3000 r.p.m. for 9 min and the concentrated buffy coats were collected. They were haemolysed with cold 0.83% NH₄Cl (adjusted to neutral pH with NaHCO₃) and then centrifuged at 800 r.p.m. for 10 min. The cells were again treated with NH₄Cl, then centrifuged as above and washed with Ham's F-12 medium (Nissui Seiyaku Co. Ltd., Japan). The purified leukocytes were suspended in the same medium containing 2% human serum at a concentration of 1 x 10⁷ cells/ml and incubated at 37 °C for 2 h. HVJ (Sendai virus) was added (final concentration 100 HAU/ml) and the leukocytes were incubated for an additional 14 to 16 h. At the end of this period the supernatant fluid was harvested and the cells were collected by centrifugation at 4200 r.p.m. for 25 min. The cells were resuspended in Ham's F-12 medium for reculturing. The recultured cells were handled in a similar manner to the first culture with modifications of cell concentration and HVJ concentration (see below). After incubation, the supernatant fluid was harvested.

Titres of IFN were determined on human amnion (FL) cells by a cytopathic inhibition microassay method with vesicular stomatitis or Sindbis virus as a challenge virus. All titres were calibrated against the international IFN-α reference (NIH G023-901-527) and are expressed as IU/ml. The human IFN-α antiserum (NIH G026-502-568) was used to neutralize IFN activity. IFN samples (approx. 200 IU/ml) were serially diluted (twofold) and each dilution was mixed with an equal volume of antiserum. After incubation at 37 °C for 60 min, the residual IFN activities were assayed as described above.

HVJ (provided by Dr T. Kishida, Cantell strain) was inoculated into embryonated eggs. Infected chorioallantoic fluids were purified by differential centrifugation and the HVJ was
Fig. 1. Effect of time of HVJ addition on IFN activity released in the second culture. HVJ was added at (a) 0, (b) 1, (c) 2 or (d) 4 h after the initiation of the incubation.

Fig. 2. Effect of HVJ concentration on IFN activity produced in the second culture.

Table 1. Effects of cycloheximide and actinomycin D on IFN yields*

<table>
<thead>
<tr>
<th></th>
<th>IFN activity (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First culture</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>56000</td>
</tr>
<tr>
<td>+ CHI</td>
<td>&lt;280</td>
</tr>
<tr>
<td>+ Act D</td>
<td>&lt;280</td>
</tr>
<tr>
<td>Second culture</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13000</td>
</tr>
<tr>
<td>+ CHI</td>
<td>13000</td>
</tr>
<tr>
<td>+ Act D</td>
<td>13000</td>
</tr>
<tr>
<td>- HVJ</td>
<td>2200</td>
</tr>
</tbody>
</table>

* Cycloheximide (CHI) and actinomycin D (Act D) were added to the medium at final concentrations of 10 µg/ml and 5 µg/ml respectively.

Table 2. Comparative yields of IFN activity

<table>
<thead>
<tr>
<th></th>
<th>IFN activity (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
</tr>
<tr>
<td>Expt. 1</td>
<td>170000</td>
</tr>
<tr>
<td></td>
<td>54000</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>100000</td>
</tr>
<tr>
<td></td>
<td>43000</td>
</tr>
<tr>
<td>Expt. 3</td>
<td>150000</td>
</tr>
<tr>
<td></td>
<td>76000</td>
</tr>
</tbody>
</table>

suspended in phosphate-buffered saline containing 5% Plasmanate (Green Cross Co. Ltd., Osaka, Japan). They were stored at −80 °C until use. To inactivate HVJ, the suspensions were exposed to u.v. radiation or to β-propiolactone treatment. The virus was irradiated in a 0.6 mm layer with u.v. (10 J/s. m²) for 2 min. The β-propiolactone solution (saline containing 0.12% β-propiolactone, 0.168% NaHCO₃ and 200 p.p.m. phenol) was added to the HVJ suspension in a
Table 3. Effect of β-propiolactone on various biological activities of HVJ

<table>
<thead>
<tr>
<th>Activity</th>
<th>Untreated</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectivity</td>
<td>$3 \times 10^{10}$ p.f.u./ml</td>
<td>$&lt;1 \times 10^2$ p.f.u./ml</td>
</tr>
<tr>
<td>Haemagglutinating activity</td>
<td>64,000 HAU/ml</td>
<td>64,000 HAU/ml</td>
</tr>
<tr>
<td>IFN-α inducing activity</td>
<td>65,000 IU/ml</td>
<td>52,000 IU/ml</td>
</tr>
<tr>
<td>IFN-α release stimulating activity</td>
<td>25,000 IU/ml</td>
<td>12,000 IU/ml*</td>
</tr>
</tbody>
</table>

*This value was the same as that obtained when no HVJ was added.

Table 4. Effect of u.v. irradiation on activities of HVJ

<table>
<thead>
<tr>
<th>Irradiation time (s)</th>
<th>Infectivity (p.f.u./ml)</th>
<th>IFN activity (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expt. 1</td>
</tr>
<tr>
<td>0</td>
<td>$3.2 \times 10^{11}$</td>
<td>36,000</td>
</tr>
<tr>
<td>15</td>
<td>$7.1 \times 10^8$</td>
<td>13,000</td>
</tr>
<tr>
<td>30</td>
<td>$1.6 \times 10^7$</td>
<td>13,000</td>
</tr>
<tr>
<td>60</td>
<td>$&lt;1 \times 10^2$</td>
<td>13,000</td>
</tr>
<tr>
<td>120</td>
<td>$&lt;1 \times 10^2$</td>
<td>13,000</td>
</tr>
<tr>
<td>240</td>
<td>$&lt;1 \times 10^2$</td>
<td>13,000</td>
</tr>
<tr>
<td>No virus</td>
<td></td>
<td>13,000</td>
</tr>
</tbody>
</table>

ratio of 1:9. After mixing, and incubation at 0°C for 10 min and at 37°C for 2 h, the suspensions were held at 4°C overnight. Infectivity of HVJ was determined on LLC-MK2 (monkey kidney) cells by plaque titration (Sugita et al., 1974).

IFN (29,900 to 30,000 IU/ml) was always obtained in the second culture of leukocytes which had been used once for IFN production. The addition of actinomycin D or cycloheximide to the second culture did not lead to the suppression of IFN production using doses which fully inhibited IFN production in the first culture (Table 1).

Fig. 1 shows the IFN yields with varying times of HVJ addition. A rapid increase of IFN activity was observed within a few minutes after the addition of HVJ, whereas IFN activity increased only gradually without the addition of HVJ. Further HVJ addition to the first culture at the end of incubation also resulted in an increase (20 to 50%) of IFN yields.

IFN yields in the second culture paralleled those obtained in the first culture (Table 2), suggesting that IFN activity in the second culture was not due to de novo synthesis, but due to the liberation of IFN which had been synthesized and accumulated in cells upon stimulation by the first addition of HVJ. More than 97% of this IFN activity was neutralized by anti-IFN-α antibody (data not shown).

As shown in Fig. 2, the IFN activity increased proportionally to the amount of HVJ added, reaching a maximum level at about 1000 HAU/ml. When HVJ (1000 HAU/ml) was added as an inducer in the first cultures, IFN production was suppressed and no IFN activity was detected (data not shown).

An attempt was made to identify the function of HVJ involved in the release of IFN. Treatment of virus with β-propiolactone resulted in loss of IFN-releasing activity together with the loss of infectivity; however, red cell agglutinating activity, IFN inducibility and cell fusion ability remained almost unchanged (Table 3). As shown in Table 4, the infectivity of HVJ decreased linearly as a result of u.v. irradiation. A 15 s irradiation period was enough to cause complete loss of the ability to release IFN. These results indicate that the IFN-releasing activity of HVJ is closely associated with HVJ infectivity.

When different inducers such as concanavalin A or phytohaemagglutinin are used, IFN-γ is produced. Similar to these inducers, the addition of HVJ caused a substantial increase in IFN-γ yields as well as IFN-α (Table 5). The protein concentration in the culture medium was quite unchanged around the period of HVJ addition. Other reagents for membrane stimulation, such
Table 5. Effect of HVJ addition on yield of IFN-γ in the second culture*

<table>
<thead>
<tr>
<th>IFN activity (IU/ml)</th>
<th>First culture</th>
<th>Second culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5000</td>
<td>800</td>
</tr>
<tr>
<td>− HVJ</td>
<td>2500</td>
<td></td>
</tr>
<tr>
<td>+ HVJ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Human leukocytes were suspended at 4 × 10⁶/ml in Ham's F-12 medium containing 2% human serum, and incubated for 2 h at 37 °C. Then concanavalin A (10 μg/ml) was added and the samples were incubated for 3 days. The second culture was performed in a way similar to the method used for IFN-α production, except that the cell concentration differed. IFN titres were determined on FL cells by a cytopathic inhibition microassay using Sindbis virus for challenge. IFN-γ titres were calibrated against the international reference (NIH G023-901-530).

As DEAE-dextran, A23187, polyethylene glycol or poly(I)-poly(C) had no effects on the increase of IFN titre.

Addition of the same amount of HVJ as that used as an inducer in the first culture produced an IFN titre from 2 × 10⁴ to 3 × 10⁴ IU/ml (15 to 20% of the yield in the first culture), while the addition of ten times more HVJ as had been used in the first induction resulted in a titre between 7 × 10⁴ and 15 × 10⁴ IU/ml, equivalent to that in the first culture. Although this method uses large amounts of HVJ for obtaining additional IFN and is therefore not practical for large-scale production, the mechanism on the stimulatory effect of HVJ on IFN release is worthy of further study.

The present study shows that in order to make the best use of human leukocytes from donated blood, cells used once for IFN-α production can be recovered and used again for IFN-α production, since the HVJ stimulated release of IFN-α already synthesized and present within the cells.

We would like to thank Mrs Y. Aoki for her contribution to the early stages of this work and Drs Y. Okubo and M. Tanaka for their support and encouragement. We also thank Drs T. Akazawa, Nagoya University, Nagoya, Japan and S. C. Huber, North Carolina State University, Raleigh, North Carolina, U.S.A., for helpful discussions and critical reading of the manuscript.

REFERENCES


(Received 5 August 1985)