The Influence of Ultraviolet-inactivated Sendai Virus on Marek’s Disease Virus Infection in Tissue Culture

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SUMMARY
The presence of ultraviolet-inactivated Sendai virus increased the transfer of Marek’s disease virus from infected to uninfected cultured chick kidney cells. This effect was seen after incubation of infected and uninfected chick kidney cells in the presence of ultraviolet-inactivated Sendai virus at 4°C. There was only a slight further increase in transfer of infection with subsequent incubation at 37°C. The close apposition of infected and uninfected cells occurring during the agglutination produced by treatment with ultraviolet-inactivated Sendai virus in the cold, rather than complete cell fusion may have been the main means by which treatment with ultraviolet-inactivated Sendai virus increased the transfer of infection.

INTRODUCTION
The infectivity of Marek’s disease virus has been shown to be strictly cell-associated in blood and tumour cells and in cultured chick kidney cells (Biggs & Payne, 1967; Biggs et al. 1968). It was postulated by Churchill (1968) that the transfer of virus from cell to cell in culture was probably not mediated by released cell-free virus but was due to an undetermined mechanism requiring cell contact. Ultraviolet-inactivated Sendai virus produces cell fusion and the formation of intercellular cytoplasmic bridges between homologous (Okada, 1962) and heterologous (Harris & Watkins, 1965) cells and activates the transfer of non-infectious virus material from infected cells to normal sensitive cells (Gerber, 1966; Svoboda, Machala & Hložánék, 1967; Vigier, 1967). I report in this paper that similar treatment with Sendai virus increases the transfer of Marek’s disease virus from infected to uninfected cultured chick kidney cells.

METHODS

Viruses

*Marek’s disease virus.* The following isolates of Marek’s disease virus were used as frozen stocks of infected cultured chick kidney cells:


(b) The GA isolate of Marek’s disease virus made from a case of acute Marek’s disease (Eidson & Schmittle, 1968).

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(c) The HPRS-24 isolate of Marek's disease virus, made from a normal chicken, which lacked pathogenicity in low tissue culture passage (P. M. Biggs & B. Milne, personal communication).

The assay technique in cultured chick kidney cells described by Churchill (1968) was used for the titration of cells infected with Marek's disease virus.

_Sendai virus_. Sendai virus (strain HvJ) was kindly supplied by Dr H. G. Pereira of The National Institute for Medical Research, London. Because Sendai virus is commonly grown in chick embryos which are not tested for contamination with avian leukosis virus, the following technique was used for the preparation of Sendai virus. Two to four haemagglutination units (HAU) of Sendai virus were used to inoculate 10-day-old Sykes line B Rhode Island Red chick embryos by the allantoic route. Sykes line B Rhode Island Reds are resistant to the infection with avian leukosis viruses of both A and B subgroups (P. M. Biggs & L. N. Payne, personal communication). The allantoic fluid was harvested 3 days later, centrifuged at 4000g for 25 min. and the HA titre determined. The harvested Sendai virus was serially transferred four times in chick embryos of the same origin using a dose of 2 HAU/embryo. After the last passage, allantoic fluid was harvested and clarified at 4000g for 25 min. The supernatant fluid was centrifuged at 22,000g for 1 hr, the virus pellet resuspended in Hanks's balanced salt solution (BSS) and the HA titre determined.

Forty and 400 HAU of stocks of Senadi virus used in the experiments were inoculated on chick embryo fibroblasts susceptible to all known subgroups of leukemia virus. Interference (Rubin, Cornelius & Fanshier, 1961) and COFAL (Sarma, Turner & Huebner, 1964) tests after three 5-day passages confirmed the absence of contamination with leukemia viruses.

_Treatment of cell mixtures with ultraviolet-inactivated Sendai virus_. Three methods were used: (a) Mixtures of freshly thawed and washed Marek's disease virus (23,000 p.f.u.) and freshly prepared chick kidney cells (2 x 10⁷) were suspended in 2 ml. of Hanks's BSS without sodium bicarbonate. The pH was adjusted to 7.6 with tris buffer. Two thousand or 4000 HAU of ultraviolet-inactivated Sendai virus (Svoboda et al. 1967) in 1 ml. of Hanks's BSS was added to 2 ml. of the cell mixture. Control cell mixtures were suspended in 3 ml. of Hanks's solution without ultraviolet-inactivated Sendai virus. The mixtures were chilled in an ice bath for 30 min. to permit the agglutination of cells and were then transferred to a water bath for 30 min at 37°C. During incubation at 37°C the mixtures were shaken for 1 min. every 10 min. To remove the ultraviolet-inactivated Sendai virus, the cells were washed by pipetting vigorously, and after resuspension in growth medium were seeded on plastic Petri dishes at the rate of 2 x 10⁶ cells/6 cm. dish. The medium was changed to maintenance medium after 3 days and the number of plaques determined 7 to 10 days after plating.

(b) Freshly thawed stocks of Marek's disease virus (46,000 p.f.u.) were washed in phosphate-buffered saline and 1000 HAU of ultraviolet-inactivated Sendai virus added. The cells were incubated under the same conditions as in (a), and both untreated cells and cells treated with Sendai virus were seeded on 3-day-old monolayers of chick kidney cells at rates of 10⁴, 2 x 10⁴, 5 x 10⁴, 10⁵ and 5 x 10⁵ cells/6 cm. Petri dish.

(c) Freshly thawed stocks of Marek's disease virus (46,000 p.f.u.) were washed in phosphate-buffered saline. The infected cells were mixed with 2 x 10⁶ fresh chick kidney cells and 2000 HAU of ultraviolet-inactivated Sendai virus were added. The mixture was treated as in (a), and the cells were seeded on 3-day old monolayers of chick kidney cells at rates of 10⁴, 5 x 10⁴, 10⁵ and 5 x 10⁵ cells/6 cm. Petri dish.
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Tissue culture media and techniques. Chick kidney cells were prepared and cultured by the methods described by Churchill (1968).

RESULTS

The toxicity of ultraviolet-inactivated Sendai virus for mixtures of freshly thawed chick kidney cells infected with Marek’s diseased virus (HPRS-16) and freshly prepared chick kidney cells was examined. Doses of 8000 and 12,000 HAU of ultraviolet inactivated Sendai virus were toxic for chick kidney cells, and subsequent cultivation of treated cells was impossible. Doses of 2000 and 4000 HAU were the most suitable. Using doses of 2000 and 4000 HAU and treatment (a) the number of specific plaques was significantly increased compared with the controls. An increase in plaque formation was also found when Marek’s disease virus was mixed with freshly prepared chick kidney cells, treated with Sendai virus and seeded on monolayers of primary chick kidney cells, as described in Methods under treatment (c). When ultraviolet-inactivated Sendai virus was added to freshly thawed stocks of Marek’s disease virus alone, as in treatment (b) no increase in plaque formation was noted.

Using treatment (a), the effect of ultraviolet inactivated Sendai virus on transmission of Marek’s disease virus was verified with other isolates of Marek’s disease virus. Several experiments were made with HPRS-16, HPRS-16/Att., GA and HPRS-24 isolates (Table 1). Specific plaques were increased by 50 to 100% when mixtures of cells infected with Marek’s disease virus and uninfected cells were treated with ultraviolet-inactivated Sendai virus. The increases were statistically significant by Student’s t test (Table 1).

To determine the effect of concentration of infected cells on transfer of infection induced by ultraviolet-inactivated Sendai virus, serial dilutions of chick kidney cells infected with Marek’s disease virus isolates HPRS-16 and HPRS-16/Att. were mixed with a standard number of fresh chick kidney cells. An increase in plaque formation in mixtures treated with ultraviolet-inactivated Sendai virus compared with controls was confirmed (Table 2). A fourfold reduction in the concentration of infected chick kidney cells did not affect the efficiency of transfer of infection induced by ultraviolet-inactivated Sendai virus.

According to Schneeberger & Harris (1966), inactivated Sendai virus particles are at first

Table 1. The effect of treatment with ultraviolet-inactivated Sendai virus on Marek’s disease virus infection

<table>
<thead>
<tr>
<th>Marek’s disease virus</th>
<th>Sendai virus HAU</th>
<th>Mean number of plaques</th>
<th>t value</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPRS-16</td>
<td>2000</td>
<td>297.30</td>
<td>8.6*</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>395.77</td>
<td>19.5*</td>
<td>1.71</td>
</tr>
<tr>
<td>HPRS-16/Att.</td>
<td>2000</td>
<td>186.00</td>
<td>1.88‡</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>238.40</td>
<td>6.00*</td>
<td>1.49</td>
</tr>
<tr>
<td>GA</td>
<td>2000</td>
<td>76.00</td>
<td>7.39*</td>
<td>1.59</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>101.20</td>
<td>4.80‡</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>6000</td>
<td>110.20</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>HPRS-24</td>
<td>4000</td>
<td>154.80</td>
<td>5.74*</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>6000</td>
<td>181.80</td>
<td>6.91*</td>
<td>1.64</td>
</tr>
</tbody>
</table>

* = P < 0.001, ‡ = Not significant.
adsorbed to and then agglutinate cells in a suspension of 4°. Cytoplasmic communication usually develops after a short incubation at 37°, while cell fusion is completed after 30 min. at 37°. Experiments were therefore made to examine the influence of time of incubation on transfer of Marek's disease virus induced by ultraviolet-inactivated Sendai virus. Treatment (a) did not increase the plaque count when the mixtures were seeded immediately after ultraviolet-inactivated Sendai virus had been added (Fig. 1a). However, there was an increase in number of plaques when the cell mixtures were incubated in an ice bath for 30 min. before seeding. Only a slight further increase in number of plaques was noted when incubation at 4° was followed by incubation at 37° for 15 or 30 min (Fig. 1a).

Table 2. The effect of ultraviolet-inactivated Sendai virus on infection with different doses of Marek's disease virus.

<table>
<thead>
<tr>
<th>Marek's disease virus</th>
<th>Estimated number of cells infected with Marek's disease virus</th>
<th>Sendai virus HAU</th>
<th>Mean number of plaques</th>
<th>t value*</th>
<th>Increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPRS-16</td>
<td>50</td>
<td>—</td>
<td>53.40</td>
<td>—</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>—</td>
<td>107.20</td>
<td>—</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>—</td>
<td>213.80</td>
<td>—</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2000</td>
<td>105.40</td>
<td>10.59</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2000</td>
<td>173.60</td>
<td>7.01</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2000</td>
<td>309.80</td>
<td>10.55</td>
<td>1.44</td>
</tr>
<tr>
<td>HPRS-16/Att.</td>
<td>28</td>
<td>—</td>
<td>NT</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>—</td>
<td>52.20</td>
<td>—</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>114</td>
<td>—</td>
<td>122.20</td>
<td>—</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>4000</td>
<td>87.80</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>57</td>
<td>4000</td>
<td>105.20</td>
<td>7.68</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td>114</td>
<td>4000</td>
<td>248.80</td>
<td>6.86</td>
<td>2.03</td>
</tr>
</tbody>
</table>

NT = not tested. * = P < 0.001.

Because treatment with ultraviolet-inactivated Sendai virus first produces agglutination of cells, experiments were undertaken to find out whether agglutination itself had some effect on the transfer of infection. Experiments were designed in which mixtures of cells infected with Marek's disease virus and uninfected cells were prepared as described under treatment (a), but phytohaemagglutinin (Wellcome, England) was used instead of ultraviolet-inactivated Sendai virus. Phytohaemagglutinin was reconstituted in 5 ml. of sterile distilled water and doses of 0.025, 0.05 and 0.2 ml./2 x 10⁷ cells were used. Doses of 0.05 and 0.1 ml. produced rapid agglutination but did not impair the growth of the cells. A dose of 0.025 ml. produced no visible agglutination, and a dose of 0.2 ml. was toxic for chick kidney cells. After incubation the cells were washed and seeded on dishes; no increase in plaque formation was observed (Fig. 1b).

To determine the possible 'helper effect' of ultraviolet-inactivated Sendai virus on the transfer of Marek's disease virus infection in tissue culture, doses of ultraviolet-inactivated Sendai virus, similar to those used in previous experiments, were added to monolayers of chick kidney cells at intervals after inoculation with cells infected with Marek's disease virus. At intervals of 1, 2, 4, 24, 48 and 72 hr after infection with Marek's disease virus, no increase or decrease in plaque counts was noted.
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Fig. 1a. The influence of different incubation times on ultraviolet-inactivated Sendai virus treated and untreated mixtures of Marek’s disease virus infected and uninfected cells. ●—●, control; ○—○, treated with Sendai virus.

Fig. 1b. The influence of phytohaemagglutinin on Marek’s disease virus infection.

**DISCUSSION**

My results show that ultraviolet-inactivated Sendai virus promotes the transfer of Marek’s disease virus from infected to uninfected cultured chick kidney cells. The specificity of Sendai virus action seems to be confirmed by the lack of effect of phytohaemagglutinin treatment on transmission of Marek’s disease virus.

Because the greatest increase in Sendai virus-induced transfer of Marek’s disease virus infection occurred after incubation at 4° alone, it is possible that Marek’s disease virus is transferred to normal chick kidney cells within the early step of cell apposition. However, because Hosaka & Koshi (1968) suggested that degradation of cell membranes and communication between the cytoplasm of adjacent cells occurs after short periods at 37° (1 to 5 min.) in the region of adsorbed Sendai virus particles, and because mixtures of cells + Sendai virus were washed in medium at 37° after incubation at 4°, this treatment may also have influenced the transfer in Marek’s disease virus infection. It is not known why Sendai virus treatment of freshly thawed stocks of Marek’s disease virus, which probably include a higher proportion of uninfected chick kidney cells, was not effective.

It appears that the dose of ultraviolet-inactivated Sendai virus as well as the number of infected cells in a mixture play a role in transfer of Marek’s disease virus infection. More plaques were found after treatment with 2000 and 4000 HAU, but higher doses, which were expected to be more efficient, could not be tested because of the toxicity of ultraviolet-inactivated Sendai virus for chick kidney cells.

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REFERENCES


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