Enhanced Production of a Human Spumavirus (Retroviridae) in Semi-permissive Cell Cultures after Treatment with 5-Azacytidine

By J. HOTTA AND P. C. LOH*

Department of Microbiology, University of Hawaii, Honolulu, Hawaii 96822, U.S.A.

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SUMMARY

Infection by a human spumavirus of human foetal diploid lung (HFDL) cells was found to be productive with virus titres ranging from $10^3$ to $10^5$ p.f.u./ml. In contrast, infection of recovered amnion (RA) aneuploid cells resulted in a persistent infection with less than 100 p.f.u./ml infectious virus produced. The decreased sensitivity of RA cells to the spumavirus was not due to the failure of virus to penetrate into the cell since infectious virus was not produced even after transfection of infectious proviral DNA. The effect of 5-azacytidine, an inhibitor of DNA methylation, on virus replication was examined. Whereas virus production in HFDL cells was not affected, there was a 100-fold increase in virus yield in RA cells treated with the drug for at least 48 h and maximum virus yields were obtained 4 days post-infection.

The human syncytium-forming virus (HSFV) is a spumavirus of the family Retroviridae (Achong et al., 1971). While the other subfamilies of the Retroviridae, the oncoviruses and lentiviruses, have been studied comprehensively in many laboratories, the spumaviruses (also called foamy viruses), in contrast, have not been characterized in detail. In light of the current concern of the role of the related subgroups (lentivirinae and oncovirinae) in malignant disease, it is important to learn more about the latent and persistent infections caused by the spumaviruses (Hooks & Detrick-Hooks, 1981; Hooks & Gibbs, 1975).

A previous report from this laboratory indicated that HSFV replicated in only fibroblast-like diploid cells, such as human foetal diploid lung (HFDL) no. 645 cells, and not in epithelial-like aneuploid cells, such as the recovered amnion (RA) cells (Loh & Ang, 1981). The semi-permissiveness of the RA cells was found not to be due to the failure of the adsorption of virus to cells (Loh & Matsuura, 1984). The present study examines the mechanisms of RA cell semi-permissiveness through transfection experiments with infectious proviral DNA of HSFV. The possible role of cellular gene methylation (Jones, 1985; Razin & Riggs, 1980) in controlling RA cell sensitivity to HSFV was also analysed by studying the effect of 5-azacytidine (5-AzaCR), an inhibitor of DNA methylation, on HSFV-infected RA cells.

Both HFDL and RA cells were grown at 37 °C in MEM supplemented with 10% foetal calf serum. Virus stocks of HSFV were prepared in HFDL no. 645 cells (Loh & Ang, 1981) and poliovirus type 1 was prepared in RA cells. HSFV was assayed in HFDL cells as previously described (Loh et al., 1977) and poliovirus type 1 was assayed in RA cells using the standard agar overlay plaque technique (Dulbecco & Vogt, 1954).

Total cellular DNA was extracted from HSFV-infected HFDL cells by a previously described modification of the Marmur (1961) method (Chiswell & Pringle, 1979). Since hydrodynamic shearing of large DNA molecules (mol. wt. greater than $30 \times 10^6$) has been shown to increase the specific infectivity of Rous sarcoma virus (Levy et al., 1974) and visna virus (Haase et al., 1976) proviral DNA by increasing the efficiency of uptake, the HSFV DNA was sheared by passing through an 18-gauge needle four times. Poliovirus RNA was extracted from whole virus preparations using the same extraction procedure.
Quantification of nucleic acids was determined by measuring $A_{260}$. Yields of DNA from four 150 cm² flasks varied from 1 to 2 mg while poliovirus (approximately $2 \times 10^{10}$ p.f.u.) yielded 400 to 600 μg RNA. The $A_{260}/A_{280}$ ratios ranged from 1.85 to 1.95.

Subconfluent cell cultures (HFDL or RA cells) were transfected with purified HSFV proviral DNA using a modification of the calcium phosphate co-precipitation technique originally described by Graham & van der Eb (1973) and Stow & Wilkie (1976). Poliovirus RNA was transfected in the same manner into RA or 3T3 cells.

5-AzaCR (Sigma) was prepared as a 2 mM stock solution and stored frozen in aliquots. Subconfluent cell cultures were treated with the drug at a final concentration of 0.2, 2, 20 or 200 μM for a period of 1 to 3 days before or after virus infection. At various time intervals post-infection (p.i.) the cells were harvested and infectious virus yields were titrated in HFDL cells.

Infection of HFDL and RA cells by HSFV confirmed previous results that HFDL cells are permissive while RA cells are semi-permissive for HSFV (Loh & Ang, 1981; Loh & Matsuura, 1984). While infected HFDL cells showed characteristic syncytia and cytoplasmic vacuolation, infected RA cells showed little or no c.p.e. Virus yields from HFDL cells at 72 h p.i. ranged from $10^3$ to $10^5$ p.f.u./ml. On the other hand, RA cells underwent a persistent infection with production of low levels of virus, less than 100 p.f.u./ml.

To determine whether the semi-permissiveness of RA cells to HSFV infection was due to a failure of the virus to penetrate into the cell, HSFV proviral DNA was extracted from permissive HFDL cells and transfected into RA cells. Early attempts to isolate free unintegrated proviral DNA by the Hirt (1967) method from HSFV-infected HFDL cells were not always successful and the yields were extremely low. Efforts were then directed towards extracting total cellular DNA from HSFV-infected HFDL cells late in the infectious cycle at which time the chances of isolating infectious DNA were greater.

The optimum conditions for transfection of HSFV proviral DNA were initially determined (Table 1). Whereas DNA extracted from uninfected cells did not produce any plaques in HFDL cells, DNA extracted from HSFV-infected cells produced an average of two plaques per 20 μg DNA. Brief treatment with 20% DMSO increased the transfection efficiency by sevenfold (15 p.f.u./20 μg DNA). The efficiency of transfection was also found to be dependent on the order of DNA addition and DMSO treatment. Treatment of cells with DMSO before DNA addition or substitution of DMSO with 30% polyethylene glycol 6000 (PEG) (Sutherland & Bennett, 1984) did not produce plaques.

The infectivity of the transfected material was found to be sensitive to DNase and insensitive to RNase and proteinase K and it was concluded that the infectious material was DNA. The syncytia formed after transfection appeared similar to those formed by the original intact virus and the progeny virus produced was infectious for HFDL cells (Table 2).

Under similar conditions, transfection of RA cells with infectious proviral DNA did not result in the production of infectious virus. One may argue that while the conditions for transfection may have been optimal for HFDL cells, they were not for RA cells. However, when poliovirus RNA was transfected into RA cells under the same conditions, infectious virus was produced (data not shown).

Gene expression in eukaryotic cells has been reported to be dependent on DNA methylation of cytosine residues. This hypothesis is based, among other results, on reports that treatment of cell cultures with 5-AzaCR causes a stable and conserved DNA hypomethylation pattern (Hsiao et al., 1984) and leads to activation of viral genes or of cellular enzyme genes (Jones, 1985; Razin & Riggs, 1980). On the basis that DNA methylation may play a role in HSFV infection the effect of 5-AzaCR on virus production in RA cells was examined.

Treatment of both HFDL and RA cell cultures 1 day after seeding with 200 μM-5-AzaCR was found to be cytotoxic. In contrast, the cell cultures treated with 20 μM or less were unaffected and their morphology, growth rate and cell viability, as measured by the trypan blue exclusion technique, were similar to untreated controls. Treatment of HFDL cells with 20 μM or less of 5-AzaCR was found to have little or no effect on virus production (Fig. 1). Virus-infected RA cells treated with 0.2 μM-5-AzaCR also showed little or no increase in virus production when compared to untreated virus-infected RA cells. In contrast, treatment with either 2 μM or 20 μM-
Fig. 1. Effect of 5-AzaCR on HSFV production. Duplicate cultures of HFDL (■) or RA (■) cells were infected with HSFV and then treated with the indicated concentrations of 5-AzaCR for a total of 48 h. At 6 days p.i. the infected cells and supernatant were harvested and infectious virus yields were titrated in HFDL cells.

Fig. 2. Kinetics of HSFV production in HSFV-infected 5-AzaCR-treated RA cells. Duplicate cultures of RA cells were treated with 2 μM 5-AzaCR either 24 h before (■) or after virus infection. Cultures exposed to 5-AzaCR after virus infection were treated for a total of 24 (○), 48 (▲) or 72 h (○). At 0, 1, 2, 4 and 6 days p.i., the infected cells and supernatant were harvested and virus yields were titrated in HFDL cells.

Table 1. Effects of adjuvants on DNA transfection

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Cytotoxicity*</th>
<th>P.f.u./20 μg DNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>Pre-treatment with PEG (30%)</td>
<td>+ +</td>
<td>0</td>
</tr>
<tr>
<td>Pre-treatment with DMSO (20%)</td>
<td>+ +</td>
<td>0</td>
</tr>
<tr>
<td>Post-treatment with DMSO (20%)</td>
<td>+ +</td>
<td>15</td>
</tr>
<tr>
<td>Post-treatment with DMSO (25%)</td>
<td>+ + + +</td>
<td>2</td>
</tr>
<tr>
<td>Post-treatment with DMSO (10%) + glucose (20%)</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

* Cytotoxicity ranged from + (light c.p.e.) to ++++ (extensive c.p.e.).
† The DNA was extracted from HSFV-infected HFDL cells. The values given are the mean of four flasks.

Table 2. Infectivity of DNA from HSFV-infected cells after different treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P.f.u./20 μg DNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recipient cells</td>
</tr>
<tr>
<td></td>
<td>HFDL</td>
</tr>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Proteinase K (50 μg/ml, 15 min at 37 °C)</td>
<td>15</td>
</tr>
<tr>
<td>DNase (50 μg/ml, 30 min at 37 °C)</td>
<td>9</td>
</tr>
<tr>
<td>RNase (50 μg/ml, 15 min at 37 °C)</td>
<td>0</td>
</tr>
</tbody>
</table>

* The values given are the mean of four flasks.

5-AzaCR, caused an enhancement of virus production of about 100-fold in RA cells. However, virus production was never as high as that found in untreated infected HFDL cells. Whether the increase in virus production was due to a few cells producing more virus or more cells in the population producing virus remains to be determined. Previous studies employing infectious
centre assays indicated that less than 10% of RA cells were producing HSFV (Loh & Matsuura, 1984).

Kinetic studies of virus production in 5-AzaCR-treated HSFV-infected RA cells indicated that enhanced virus production was seen only in cells treated for at least 48 h and maximum virus yields were observed 4 days p.i. There was no increase in virus titres with treatment longer than 48 h. Virus production in cells treated with either 5-AzaCR before virus infection or in cells treated with 5-AzaCR for only 24 h after infection was no different to that in untreated virus-infected RA cells (Fig. 2).

In conclusion, the DNA transfection experiments performed in the present study clearly indicated that resistance to HSFV infections was due to an intracellular event, as transfection into HFDL cells, but not RA cells, yielded infectious virus. Additional experiments with 5-AzaCR strongly suggested that DNA hypomethylation may play an important role in the establishment of the permissiveness of RA cells to HSFV. Whereas treatment with the drug had little effect on HSFV production in HFDL cells, treatment of virus-infected RA cells with 2 μM-5-AzaCR for at least 2 days increased virus production by 100-fold at 4 days p.i. Further examination of the effects of 5-AzaCR on HSFV-infected RA cells could be useful in studying the relationship between spumavirus latency and/or persistence and DNA methylation.

REFERENCES


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