Persistent Infection with Infectious Pancreatic Necrosis Virus Mediated by Defective-interfering (DI) Virus Particles in a Cell Line Showing Strong Interference but Little DI Replication

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SUMMARY

The characteristics of chinook salmon embryo cells persistently infected with infectious pancreatic necrosis virus were consistent with defective-interfering (DI) particle-mediated persistence. All the cells were infected and were slowly releasing virus, but they could be cured of virus in the presence of antiserum. Immunofluorescence showed that the amount of virus antigen in persistently infected cells was low. This fact, coupled with the observation that few DI particles were released by these cells, indicated that DI particles were not replicated to excess in this cell line.

INTRODUCTION

Infectious pancreatic necrosis virus (IPNV) of fish is the prototype of a new group of viruses whose members contain two segments of double-stranded RNA and have a naked single-shelled icosahedral structure. IPNV exhibits an autointerference phenomenon (Nicholson & Dunn, 1974) that has been linked to the presence of an interfering virus particle (Macdonald & Yamamoto, 1978). The interfering factor co-purified with standard virus, but was enriched in the region immediately above standard virus in a shallow CsCl gradient, which showed that the factor was distinct from standard virus and was defective in replication. It conferred on recipient cells, with apparent one-hit kinetics, the ability to survive infection by normally cytolytic standard IPNV (Macdonald & Yamamoto, 1978).

Huang & Baltimore (1970) have defined defective-interfering (DI) virus particles as deletion mutants that contain normal viral structural protein and, in addition, that require (and also inhibit) the standard virus during their replication. Because of the difficulty in purifying the IPNV factor free of standard virus, it has proved impossible to determine whether it conforms to all the properties of a DI particle as defined above. Thus, although the IPNV interfering particle is undefined in molecular terms, it will be called a DI particle in this report because of its biological properties.

Recently, Holland et al. (1980) have reviewed a number of different DI particle-induced persistent infections of vertebrate cells in culture. A number of properties were shared by these persistently infected cells, and Holland et al. (1980) proposed a set of defining characteristics for persistent infections induced and maintained by DI particles: (i) all or nearly all of the cells are infected at all times; (ii) persistence endures for years and is stable at the individual cell level with few crises; (iii) antibody cures only when the cells are well isolated; (iv) the cells are resistant to homologous virus and are fully susceptible to heterologous viruses; (v) interferon is never detected; (vi) DI nucleocapsid accumulates inside the cell in large amounts; (vii) cloned standard virus from the culture is unable to induce persistent infection without DI particles or interferon (at least during the initial stages of persistence).
IPNV is able to initiate a persistent infection of fish cells in culture (Macdonald & Yamamoto, 1978; Ahne, 1978; Hedrick et al., 1978; Macdonald & Kennedy, 1979) mediated by the IPNV DI particle discussed above. The chinook salmon embryo (CHSE-214) cells that we have used have a defect in the interferon system (Macdonald & Kennedy, 1979), show extreme cytopathology and death in the presence of standard virus, yield >1000 plaque-forming units (p.f.u.) per cell progeny virus during lytic infection, and are the most sensitive cell line for IPNV plaque assay (R. D. Macdonald, unpublished observations). Some serotypes of IPNV formed ringed plaques on CHSE-214 cells because they generated many DI particles, and some serotypes formed clear plaques by generating few DI particles (Macdonald, 1978; Macdonald & Gower, 1981). We have initiated several IPNV persistently infected CHSE-214 cell lines using either a high DI-producing serotype (OV-3) or a low DI-producing variant of a different serotype (Jasper virus, JV) in order to determine the major characteristics of the virus/cell interaction during long-term persistence. With the exception of a few quantitative differences, we have found that the characteristics of cells persistently infected by either serotype of IPNV conformed to the criteria set forth by Holland et al. (1980) for persistent infection involving DI particles.

METHODS

Cells and viruses. The chinook salmon embryo cell line (CHSE-214) was obtained from Dr J. L. Fryer (Oregon State University, Corvallis, Oregon, U.S.A.). The fathead minnow cell line (FHM) was obtained from the American Type Culture Collection (ATCC, Rockville, Md., U.S.A.). Cells were grown in Eagle's minimal essential medium (MEM) containing 100 IU/ml penicillin, 100 μg/ml streptomycin, 0.15% sodium bicarbonate and 5% foetal bovine serum at 18 °C under an atmosphere of CO₂ in air which maintained the pH of the medium at 7.4 to 7.6. Cell lines were subcultured every 7 to 14 days. The IPNV serotypes, Jasper virus (JV) and oyster virus (OV-3), were obtained from Dr T. Yamamoto (Department of Microbiology, University of Alberta, Alberta, Canada). Infectious haematopoetic necrosis virus (IHNV) and frog virus 3 (FV-3) were obtained from the ATCC.

Initiation of persistently infected cell cultures. A sample of OV-3 was plaque-purified three times and passed once in CHSE cells at a low multiplicity. CHSE cells were then infected with this sample at 2 p.f.u./cell. After incubation for 4 days, cytopathic effect (c.p.e.) had destroyed 50% of the cells. The medium was removed, the culture was washed and fresh medium was added. Within 4 days the surviving cells had grown to confluence. This cell line, designated COV79 (for CHSE cells persistently infected with OV-3, 1979), was maintained for the duration of this study and was the major cell line used for characterization. Similarly, CHSE cells were infected with a high multiplicity of JV (150 p.f.u./cell). The surviving cells (2 to 10% of the monolayer) reached confluence in 10 days and this cell line was designated CJV79. The cell lines COV80 and CJV80 were prepared in a similar manner at a later date.

Virus assay. The amount of IPNV was measured by the plaque assay described by Chang et al. (1978). In the case of the heterologous viruses (IHNV and FV-3), which do not easily plaque on CHSE cells, and in some selected experiments with IPNV, the 50% tissue culture infectious dose (TCID₅₀) method of Karber (1931) was used to assay the viruses. Confluent monolayers of CHSE cells were formed in each of 96 wells in a cluster dish. The monolayers were infected with 25 μl of half-log₁₀ or log₁₀ dilutions of the virus. The dishes were incubated at 18 °C for 7 to 21 days or until c.p.e. was complete. When IHNV and FV-3 were assayed in the presence of IPNV (as, for example, when they were used to infect persistently infected cells), 1% anti-IPNV serum was incorporated in the assay. This antiserum totally neutralized IPNV and allowed the independent assay of the heterologous viruses. The method used to measure the number of DI particles was developed by
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Macdonald & Yamamoto (1978), and depended upon the measurement of the number of cells spared from the cytolytic action of IPNV.

**Efficiency of plating and growth rates of persistently infected cells.** During a routine subculture, the number of resuspended cells/ml were counted in a haemocytometer and the number of cells that attached to the dish were counted after 24 h incubation. The efficiency of plating was expressed as the percentage of cells attached to the dish. Growth rates were determined by plating the cells into replicate dishes at equal density. Every 36 to 48 h, one dish of each cell type was fixed and stained. Dishes were counted under a light microscope, averaging 10 fields/dish. Values were expressed as cells/dish.

**Infectious centre assay.** CHSE cells lytically infected with JV (40 p.f.u./cell) assayed 4 h post-infection were used as a positive control. Such cells should score as 100% infectious centres if cell counts and plaque counts are consistent. Monolayers were washed five times with complete medium, trypsinized into 10 ml medium and washed five more times by centrifuging the cells to a pellet and resuspending in fresh medium. The concentration of washed cells was measured using a haemocytometer, averaging 12 determinations. The cell preparation was serially diluted by 10-fold dilutions and the infectious centres/ml were measured by one of two methods outlined below. (i) Plaque method: 100 μl of each virus dilution was added to a 60 mm dish of CHSE cells. The remaining procedure followed that reported earlier for a plaque assay. Plaques were counted after 3 days. Each plaque represented one infectious centre. (ii) Endpoint dilution method: 25 μl of each dilution was added to 12 replicate wells in a cluster dish containing a monolayer of CHSE cells. After 14 days, the wells were scored as either ‘+’ or ‘−’ for c.p.e. The number of infectious centres could be determined using the Poisson formula, \( m = -\ln[P(0)] \), where \( m \) = average number of infectious centres per inoculum volume and \( P(0) \) = fraction of wells receiving no virus.

Each measurement was corrected for virus released from the cells during the manipulations. This measurement was taken after the inoculation of the cells and was performed by centrifuging the cells in each dilution tube to a pellet and inoculating 25 μl of the supernatant from each tube into a cluster well containing CHSE cells. The correction factor never amounted to more than 10% of the infectious centres.

**Immunofluorescence.** Cells were washed with Hanks' balanced salt solution (HBSS) and fixed with 3.7% formaldehyde, 0.5% Triton X-100 in HBSS. The cells were then washed in distilled water and treated with 0.05% sodium borohydrate (two washings of 10 min each). Fixed cells were rinsed in phosphate-buffered saline (PBS; 0.85% sodium chloride, 0.091% potassium phosphate monobasic, 0.76% sodium phosphate dibasic pH 7.5). The PBS was removed and 0.1 ml of a 1/20 dilution of anti-OV-3 serum (which had been pre-adsorbed with uninfected CHSE cells) was added to each dish and incubated for 30 min at room temperature. After washing, the second antiserum (fluorescein-conjugated goat anti-rabbit antibodies) was added for 30 min at room temperature. The cells were mounted in 90% buffered glycerol and observed with a darkfield condenser under u.v. light in a Wild M-20 microscope. Photographs were taken on Kodak Tri-X Pan film.

**RESULTS**

**Initiation of persistently infected cells**

Persistent infection could be readily established in CHSE cells with the OV-3 serotype of IPNV. Characteristically, 50% of the cells survived infection and these cells could be cultivated into a persistently infected cell line (COV79). The number of cells that survived infection was multiplicity-dependent (Macdonald & Yamamoto, 1978); with undiluted OV-3 samples it equalled 100%, and with dilute samples (m.o.i. <0.01) it was as low as 2% or less.
Fig. 1. DI particles do not stop attachment of standard virus. A sample of standard JV was added to CHSE cells at time zero; at various times thereafter a sample of OV-3 DI particles (plus more standard virus) was added. The action of the DI particles in initiating cell survival was measured at each point. The arrows indicate cell survival values when infected with either (1) OV-3 or (2) JV alone.

Fig. 2. Growth curves for CHSE (△), COV79 (passage 32; ○) and cured (○) cells in a 21.2 cm² dish.

The JV persistently infected culture (CJV79) was established with a plaque-purified, low multiplicity virus stock. Because of this passage history, and because JV is a clear plaque (low DI) strain, only 2 to 10% of the cells survived infection and these cells were cultivated and became the cell line CJV79. Even this low DI-producing strain which had been repeatedly clonally isolated always showed some surviving cells, confirming previous studies which showed that DI particles were generated rapidly at a low level in IPNV stocks (Macdonald, 1978).

Although it has been impossible for us to clone CHSE cells, we probably were not selecting for a resistant cell type present at low levels within the population because of the fact that cell survival was multiplicity-dependent and could be as high as 100%, and because cells cured of IPNV by antiserum were again susceptible to IPNV lytic infection (see below). Cytopathic effect in IPNV persistently infected cells due to the carried virus (i.e. cell crisis) was never observed during the lifetime of the cultures. The persistently infected and uninfected cell cultures behaved and were treated in an identical fashion.

**DI particles do not stop attachment of standard IPNV**

In order to see whether the IPNV DI particles interfered at the attachment stage, or at a later intracellular step in replication, we determined whether DI particles could be added after standard virus. Cells were first infected with standard virus (JV) at 4 p.f.u./cell, and at various times post-infection, DI particles (OV-3) were added. JV infection alone yielded a survival rate of 2%; OV-3 alone yielded 35% cell survival (Fig. 1). The time in the replication cycle when DI particles act should correspond to the timing of the drop in the percentage survival curve. The results showed that there appeared to be an exponential decrease in cell survival as the addition of DI particles was delayed (Fig. 1). In particular, DI particles could be added a few hours after standard virus and still exert their full interference effect. Thereafter, the addition of DI particles was accompanied by a corresponding decrease in cell survival.
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Fig. 3. Immunofluorescent detection of IPNV protein antigen in persistently infected cells using antiserum against virions. (a) COV79 cells (passage 36), phase contrast; (b) COV79 cells, immunofluorescence of same field; (c) CHSE cells lytically infected with IPNV, phase contrast; (d) CHSE cells lytically infected with IPNV, immunofluorescence of same field; (e) normal CHSE cells, phase contrast; (f) normal CHSE cells, immunofluorescence of same field.

Growth and morphology of persistently infected cells

It was difficult to distinguish morphologically between a culture of persistently infected cells and one of uninfected cells (compare Fig. 3a with 3e). The COV79 carrier cells seemed to occupy more area when attached to the dish, which may have accounted for the observa-
tion that these cells never reached a final density equal to that attained by CHSE cells (see below). Cell viability was determined during the disruptive treatment regime of trypsinization and subculture; 93% of the CHSE cells remained viable and attached to the dish compared to 92% for COV79 cells. Growth curves for COV79 cells and CHSE cells were similar during the first 10 days after subculturing (Fig. 2). The final density attained by COV79 cells was $2.8 \times 10^5$ cells/cm$^2$ and the final density of the normal CHSE cells was $8.7 \times 10^5$ cells/cm$^2$.

All persistently infected cells contained and released virus

During the lifetime of the COV79 culture, extracellular virus could always be detected in the culture fluid in concentrations of approx. $10^4$ to $10^7$ p.f.u./ml (data not shown). There seemed to be a slight trend of decreasing extracellular virus with increasing passage number.

The virus released from the cultures could have been released from a few cells undergoing a lytic cycle, or from all the cells at a low level. Two methods were used to determine the percentage of cells in the persistently infected cultures which were releasing virus. Using the plaque method, our estimations showed that only 3 to 5% of COV79 cells scored as infectious centres and hence were releasing virus. This result was at variance with the immunofluorescent results which showed that every cell had virus antigen (Fig. 3) as well as the observation that no detectable cell death occurred after IPNV superinfection. Since it was possible that virus release was a very infrequent event, we measured infectious centres by a limiting dilution assay that could measure virus released over a period of 14 days. The data showed that a COV80 (passage 0), a COV80 (passage 7) and a COV79 (passage 36) carrier culture yielded 105%, 103% and 104% infectious centres respectively when compared to lytically infected CHSE cells run in parallel and normalized to 100%.

The discrepancy in the results between the two methods can be explained by the different incubation times inherent in the methods. The plaque assay involved an incubation of only
3 days; the limiting dilution assay was incubated for 2 weeks. The plaque assay tests can be interpreted to indicate that only 3 to 5% of the cells released virus within 24 h (since it takes 2 days to form a visible plaque), but 100% of the cells released virus within 2 weeks in the limiting dilution assay. We propose that the limiting dilution assay detected cells that were slowly releasing virus. The idea that persistently infected cells were releasing virus at very low levels was substantiated by direct measurement of the kinetics of virus release (Fig. 4). Persistently infected cells were washed free of extracellular virus and the accumulation of virus into the medium was measured. During the initial 4 days, the virus was released at a linear rate of 2.5 p.f.u./10^4 cells/day. The results from this experiment cannot be used to quantify the release of virus during the limiting dilution assay (because of the use of different washing and incubation techniques), but they did confirm that production of mature standard IPNV from persistently infected cells was slow.

The possibility that DI particles were being released from persistently infected cells was also considered, and the following evidence suggested that DI particle production was low. Firstly, addition of undiluted medium from persistently infected cells to a monolayer of CHSE cells resulted in massive c.p.e. in the indicator cells equivalent to that caused by a high dilution of standard virus in the absence of DI particles. Secondly, we noted that total virion physical particle production (standard plus defectives) was low from persistently infected cells, since no particles could be visualized as a band on a CsCl gradient after being purified from a standard (small) volume of medium. The same volume of medium from lytic infection yielded a virus band. However, by increasing the number of cells and the corresponding amount of medium, it was possible to increase the concentration of virus from persistently infected cells to enable the detection of DI particles by a cell-sparing assay that had a sensitivity of about 5 x 10^3 DI/ml (Macdonald, 1978). Such a test on COV79 cells showed that the medium contained about 0.5 DI/p.f.u. and the cells (after Freon extraction) yielded 0.7 DI/p.f.u. These values fell within the range of 0.1 to 1.0 DI/p.f.u. found for the level of DI particles produced during multiple cycles of lytic infection (Macdonald & Yamamoto, 1978) and hence did not represent an enrichment of DI particles during persistence.

**Immunofluorescence**

The detection of intracellular virus antigen using immunofluorescence is illustrated in Fig. 3. Uninfected CHSE cells showed no fluorescence, demonstrating the extremely low level of background with this technique. Lytically infected CHSE cells showed typical abundant fluorescence within the cells (Fig. 3 d) and the COV79 persistently infected culture exhibited fluorescence at a very low level in every cell (Fig. 3 b). These observations confirmed the results from the infectious centre assay that every cell (or nearly every cell) contained virus.

**Effect of superinfection on persistently infected cells**

The sensitivity or resistance of persistently infected cells to superinfection was tested by two methods. Firstly, homologous and heterologous viruses were inoculated on to cultures of persistently infected and control CHSE cells and the yield of progeny virus was measured after multiple cycles of infection and maximum cell death. Typical results are given in Table 1. Superinfection of COV79 and CJV79 cells with homologous viruses failed to increase the amount of virus in the culture above control (non-superinfected) levels, which were about 1% of the level of a lytic yield. The replication of heterologous viruses (IHNV and FV-3) as measured by yield/cell showed a replication equal to that in control CHSE cells (Table 1). In the second method, persistently infected cells were used as a host cell line for the titration of various samples of homologous and heterologous viruses. Because IHNV and
Table 1. *Superinfection of persistently infected cells with homologous and heterologous viruses*

<table>
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<tr>
<th>Superinfecting virus</th>
<th>Cell</th>
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<th>JV</th>
<th>OV-3</th>
<th>FV-3</th>
<th>IHNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHSE</td>
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<td>9.0</td>
<td>9.3</td>
<td>6.9</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>COV79 (passage 11)</td>
<td></td>
<td></td>
<td>(0.11)**</td>
<td>(0.10)</td>
<td>(0.14)</td>
<td>(0.14)</td>
</tr>
<tr>
<td>CJV79 (passage 10)</td>
<td></td>
<td></td>
<td>(0.16)</td>
<td>(0.11)</td>
<td>(0.15)</td>
<td>(0.09)</td>
</tr>
</tbody>
</table>

*Yield of virus is expressed as log TCID*<sub>50</sub>/10<sup>6</sup> cells.*

**Numbers in parentheses are standard deviation calculated according to Karber (1931).**

FV-3 formed plaques only with difficulty, we used a limiting dilution assay. When homologous IPNV was titrated on COV79 and CJV79 cells, no c.p.e. was observed (confirming that most or all of the cells were resistant to superinfection) and, thus, no titre could be determined for these virus samples. With persistently infected cells as a host, the heterologous viruses caused c.p.e. in a consistent manner so that a titre could be determined for each sample. Interestingly, the titres were significantly less than those found for the same virus samples assayed at the same time on control CHSE cells (data not shown). Since this assay measured a different parameter than the previous (yield/cell) assay, our results suggest that there was a 6- to 60-fold increase in the number of abortive infections with heterologous viruses used at low m.o.i. in IPNV persistently infected cells.

Effect of antiserum on persistently infected cells

Holland *et al.* (1980) have observed that their persistently infected cells could be cured of virus infection only with difficulty by incubation with antiserum. In our system, COV79 cells could be completely cured of virus when cultured in the presence of anti-OV-3 serum. Fig. 5 shows the progressive decline in cell-associated virus in cells cultivated in 1% antiserum. Each time the cells were subcultured (Fig. 5, arrows), culture fluid was tested for virus to ensure that the antiserum was in excess so that no extracellular infectious virus remained. Curing began within 24 h and, after 54 days of treatment, removal of antiserum was not accompanied by cell death or c.p.e.; by all criteria these cells were cured of IPNV. These cured cells were almost fully sensitive to IPNV infection, although they consistently permitted about 80% of the plaquing efficiency of control CHSE cells and yielded only 2.5 x 10<sup>3</sup> p.f.u./cell of IPNV compared to 5.7 x 10<sup>3</sup> p.f.u./cell in a normal yield. In addition, these cells maintained the lower cell density plateau characteristic of the persistently infected cells (see Fig. 2).

Lack of phenotypic alteration of the carried virus

Three traits were examined to determine if any phenotypic alterations of the virus had occurred during persistent infection.

Plaque morphology (see Macdonald, 1978, for photographs)

The OV-3 used to initiate COV79 cells yielded 100% ringed plaques (123 out of 123). The virus released from passage 42 of this persistently infected cell line also yielded 100% ringed plaques (131 out of 131). The plaque morphology of JV used to initiate CJV80 cells was predominantly clear plaque; only 3% of the plaques were ringed (4 out of 133). The virus released from CJV80 cells, passage 3, yielded 9% ringed plaques (15 out of 159).
Host range

JV, but not OV-3, was able to plaque on fathead minnow (FHM) cells (Macdonald & Gower, 1981). When a comparison of the host-range of the initial and released virus was undertaken, we found that JV continued to plaque on FHM cells and OV-3 failed to plaque with no significant difference between initial and released virus. In a clone-by-clone analysis of JV released from persistently infected cultures at passage 15, one out of 24 clones was a mutant that was unable to plaque on FHM cells (called J15, Macdonald & Gower, 1981). Since it is unclear why a host-range mutant for another cell type should be involved in persistent infection of CHSE cells, and since this mutant can also arise during lytic infection (Scherrer & Cohen, 1975), we do not consider it to be significant to our persistent infection.

Temperature sensitivity

Both initial and released virus samples from COV79 and CJV90 failed to show any temperature-sensitive (ts) mutants that were unable to replicate at a non-permissive temperature of 24 °C (Macdonald & Dobos, 1981). The virus was tested by a clone-by-clone analysis that involved either picking plaques for subsequent testing or by a plaque enlargement (temperature jump) method. In addition, when cultures of persistently infected cells were incubated at 14 °C or 24 °C instead of the usual 18 °C, there was no development of c.p.e., indicating that ts mutants were unlikely to be involved in maintaining persistence.

DISCUSSION

The characteristics of the IPNV persistently infected cells were consistent in all regards with the characteristics of DI-mediated persistence of other viruses (Holland et al., 1980). In particular: (i) all of the cells were infected by IPNV at all times, as shown by their resistance to homologous superinfection, the infectious centre assays, and the immunofluorescent results; (ii) persistence endured for at least 18 months with no cell crisis due to the carried virus, although the level of extracellular virus and intracellular virus (not shown) slowly declined with the age of the culture which may have reflected a long-term spontaneous curing process; (iii) antibody cured individual cells readily, although it required 54 days to totally eliminate virus from the culture; (iv) the cells were resistant to superinfection by homologous virus by any method used, and were fully sensitive to superinfection by heterologous viruses when the yield/cell was analysed (however, the cells did not serve to titrate the viruses at limiting dilution as efficiently as did normal CHSE cells); (v) interferon was never detected and CHSE cells appeared to have a defect in the interferon system (Macdonald & Kennedy, 1979); (vi) nucleocapsid antigen was detected in persistently infected cells at very low levels, suggesting that neither standard virus nor DI particles replicated to excess in these cells; (vii) most cloned standard virus from the persistently infected culture showed no change in phenotype from the initial virus, and could not re-establish persistent infection in the absence of DI particles. With the exception of some minor quantitative differences, we conclude that our observations were consistent with the IPNV interfering factor being a DI particle, and that cells persistently infected by the action of this DI particle behaved qualitatively in all respects like classical DI particle-mediated persistently infected cells.

However, there are some differences (the amount of intracellular virus antigen and the ease of curing with antibody) between the IPNV system and the system as it has been defined for classical DI particles. We propose that the replication of DI particles in the IPNV/CHSE cell system was not necessary for the interference of standard virus, and base this conclusion in part upon the observation that DI particles never became enriched in CHSE cells during interference (Macdonald & Yamamoto, 1978) and in part upon the observation that IPNV persistently infected cells contained very low amounts of intracellular virus protein antigen.
(Fig. 3) and no detectable dsRNA antigen (Macdonald, 1980) compared to lytically infected cells. In confirmation of this conclusion, direct measurements of DI particle activity in extracts from persistently infected cells showed only 0.5 to 0.7 DI/p.f.u.; this value fell within the range of values observed in virus samples from lytically infected cells (Macdonald & Yamamoto, 1978). These results showed that the DI particles of IPNV did not become enriched in CHSE cells even when they were given months to replicate during persistence.

There are reports of other systems that have shown a separation of DI particle replication and its interfering activity. One cloned line of HeLa cells did not replicate vesicular stomatitis virus (VSV) DI particles, but it did allow interference of standard virus by exogenous DI (Holland et al., 1976). The same cell line behaved similarly towards Semliki Forest virus (Stark & Kennedy, 1978). Similarly one line of L cells allowed VSV DI interference without replication and/or generation of DI particles (Potter & Stewart, 1976). Even in cells that were permissive for VSV DI interference and replication, it has been shown that conditions of maximum interference (using high numbers of DI particles) were accompanied by a lack of replication of the DI particles (Stampfer et al., 1969).

The simple replicative competition model proposed to explain DI particle interference (Huang & Manders, 1972; Perrault & Holland, 1972) could not have predicted these results. Whether or not such a competition model is correct, the above examples of the separation of the replication and interfering activity of well-characterized DI particles indicate that a similar effect in the IPNV/CHSE cell system is still consistent with the action of a classical DI particle in a select situation. We suggest that the CHSE cells used in our study were a poor host for DI replication although they generated DI particles at a very high rate (relative to VSV) and allowed their interfering activity. Only by postulating this host cell phenotype are we able to explain the relatively high level of interfering particles in IPNV preparations which showed little or no additional replication and enrichment. These DI particles may have been so inhibitory to their helper virus replication that they received insufficient ‘help’ to replicate themselves in this cell line.

In general, enveloped RNA viruses have been most prone to establish persistent infection and there are few model systems available for the study of persistent infection of non-enveloped RNA viruses in permissive cells. The IPNV/CHSE cell system provides such a model system, and furthermore, our study shows that the presence or absence of a virion envelope is not an important distinction in the application of the seven properties of DI-mediated persistent infection given by Holland et al. (1980), since both IPNV and enveloped viruses show these properties.

After this manuscript was prepared, a report by Hedrick & Fryer (1981) was published that confirms our conclusions that CHSE-214 cells persistently infected with IPNV replicate DI particles poorly.

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