Preliminary Characterization of a Persistent Infection of HeLa Cells with Human Rhinovirus Type 2

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SUMMARY

We were able to initiate a persistent infection (PI) in HeLa cells with a temperature-sensitive (ts-) mutant of rhinovirus type 2 (TS-1), but not with the corresponding wild-type (wt) virus. The ability to initiate a PI may be related to the multiplicity of infection. Persistence was established at 37 °C but not at 32 °C and the virus isolated from the PI was no longer temperature-sensitive. Infectious virus was continually produced at low levels throughout the course of the PI and cell cultures underwent multiple episodes of partial destruction (crisis) and subsequent recovery. PI virus and the initiating virus were neutralized to the same extent by hyperimmune polyclonal TS-1 antiserum indicating that no significant change had occurred with respect to serological type. The presence of either interferon or virus-related interfering activity could not be demonstrated in the PI cultures. Superinfection experiments in cells that were 'cured' of PI virus indicated the selection of a cell population during persistence that could no longer support the growth of homologous-type virus. This effect became less pronounced upon further passage of the cured cells. When compared with the wt and ts viruses, the PI virus yielded comparable amounts of infectious virus in HeLa cells but with decreased synthesis of RNA.

INTRODUCTION

Persistent viral infections of cells in culture have been used as a means of studying virus–host cell interactions and as model systems in attempts to elucidate mechanisms of viral persistence in vivo. Several mechanisms, exclusive of the integration of genetic material, appear to be involved in the persistence of viruses in cell cultures. Previous studies have shown that these include the production of defective interfering (DI) particles, temperature-sensitive mutants (ts-), and interferon activity (for reviews, see Friedman & Ramseur, 1979; Rima & Martin, 1976; Preble & Youngner, 1975). Each mechanism may be involved separately or in combination with the others, and frequently the factor responsible for the maintenance of the persistent infection (PI) is different from that responsible for initiation.

DI particles have been implicated in both the establishment and maintenance of PIs of LLC-MK2 cells by rubella virus (Norval, 1979) and of chinook salmon embryo cells by pancreatic necrosis virus (Kennedy & Macdonald, 1982). Ahmed & Graham (1977) have reported the increased production of DI particles associated with a PI initiated in L cells by a reovirus ts- mutant. Interferon has been used to initiate a PI in L cells with vesicular stomatitis virus (VSV) (Ramseur & Friedman, 1978) and has been shown to be responsible for the maintenance of both Semliki Forest virus/mouse L929 cell (Meinkoth & Kennedy, 1980) and Newcastle disease virus/L cell PI systems (Thacore & Youngner, 1969). Several investigators have shown that coinfection of various cell lines with a wild-type (wt) virus and its corresponding ts- mutant results in the establishment of a PI due to interference by and selection for growth of the ts- mutant (Youngner & Quagliana, 1976; De & Nayak, 1980; Sato et al., 1981). Natural selection of ts- virus in PI systems initiated with wt virus has been reported as the mechanism by which
persistence is maintained in several PI systems (Williams et al., 1981; Preble & Youngner, 1972; Hodes, 1982). In addition, the selection of viruses with ts+ phenotypes in PI systems initiated by ts− mutants has also been shown to occur (Pringle et al., 1978; Atkins, 1979). Unknown host cell factors (May & Menna, 1979; Wild & Dugre, 1978) and the temperature-dependent differential expression of viral structural proteins (Fisher & Rapp, 1979) also have been implicated in the maintenance of still other PI systems.

Infection of permissive cell lines by picornviruses normally results in the production and release of infectious virus and lysis of the host cell. Ackermann (1956) has demonstrated the ability of poliovirus to persist through many subcultures of HeLa cells in the presence of immune serum. Takemoto & Habel (1959) described a virus-cell relationship in carrier culture of HeLa cells infected with Coxsackie A9 virus. The insensitivity of HeLa cells to Coxsackie A9 virus infection and the rapid thermal inactivation of the virus were cited as the principal mechanisms for the maintenance of the carrier state. More Recently, Gauntt (1979) reported the establishment of a short-term carrier state in HeLa cells by rhinovirus type 14 in the presence of guanidine, an inhibitor of viral replication. The present study is the first report of the establishment and characterization of a long-term persistent infection in the permissive HeLa M cell line by human rhinovirus type 2 (HRV-2) without the use of inhibitors and in the absence of virus-specific antibody.

**METHODS**

**Cell cultures, viruses and antisera.** HeLa cells (Hamparian, 1979) were grown in Eagle's MEM containing final concentrations of 10% foetal bovine serum (FBS), 0.08% NaHCO₃ and 5 μg/ml gentamicin. For maintenance, the serum was reduced to a final concentration of 2% and the NaHCO₃ was increased to 0.16%. The prototype strain (HGP) of HRV-2 was from the viral stocks of this laboratory. Poliovirus type 2 (PV-2) and VSV were provided by Dr J. H. Hughes. Seed viruses were propagated in HeLa cells and partially purified by fluorocarbon (trichlorotrifluoroethane) extraction. The isolation and characterization of HRV-2 ts− mutants (permissive temp., 32 °C; restrictive temp., 37 °C), designated TS-1 to TS-5, have been reported elsewhere (Evans et al., 1980). Antisera for the HRV-2 and TS-1 viruses were prepared in guinea-pigs as described previously (Hughes et al., 1974).

**Quantification of viruses.** The methodology used for plaque assays has been described in detail elsewhere (Conant & Hamparian, 1968). HeLa cell cultures prepared either in 3 oz prescription bottles or six-well cell culture plates (Costar) were used. Each tenfold dilution of virus was inoculated in duplicate, 1 ml into bottle cultures and 0.5 ml into wells. Following an adsorption period of 90 min at 37 °C, the cultures were overlaid with maintenance medium containing 0.7% agarose. After 4 days of incubation at either 32 °C or 37 °C for rhinoviruses and 48 h at 37 °C for PV-2 and VSV, a second overlay was added consisting of maintenance medium containing 1% agarose and 0.35% neutral red. Plaques were scored after overnight incubation in the dark.

**Serum neutralization.** Antiserum to TS-1 was serially diluted and 0.5 ml of each dilution was mixed with 0.5 ml of maintenance medium containing 100 TCID₃₀ of the virus to be tested. Following 1 h incubation at room temperature, 0.2 ml of each serum–virus mixture was inoculated into each of four tube cultures of HeLa cells. The tubes were rolled at 32 °C and scored daily for c.p.e. The 50% endpoint of neutralization was calculated by the method of Reed & Muench (1938).

**Establishment of persistently infected cultures.** To initiate PI, confluent HeLa cell monolayers devoid of medium were inoculated with 0.01, 0.001 or 0.0001 p.f.u./cell. Following 90 min of adsorption at 32 °C, 10 ml of maintenance medium was added and the cell cultures were incubated at either 37 °C or 32 °C. Cultures were observed daily for c.p.e. and were subcultured every 7 to 8 days if an adequate number of viable cells were present. If the amount of c.p.e. was too extensive, the cultures were re-fed intermittently until subculturing at a ratio of 1:4 could be accomplished. Weekly samples of culture supernatants were stored at −20 °C until used for infectivity assays and other studies.

**Infectious centre assay.** Persistently infected cells were trypsinized, suspended in growth medium containing 100 units of antibody to the TS-1 virus (1 unit neutralizes 100 TCID₃₀ of TS-1 virus) and incubated at 37 °C for 1 h. Subsequently, the cells were washed three times with maintenance medium and collected by centrifugation at 400 g. The number of viable cells (trypan blue exclusion) were determined, tenfold dilutions of the cell suspension were made in maintenance medium and a 0.5 ml aliquot of each dilution was plated onto duplicate cultures of HeLa cells. Following a 90 min adsorption period at 32 °C, the cultures were overlaid and handled as described under *Quantification of viruses*. The percentage of infectious centres was calculated on the basis of viable cell counts.
Superinfection experiments. Duplicate 3 oz bottle cultures of uninfected and persistently infected HeLa cells were inoculated with each of the following viruses: TS-1, HRV-2, HRV-4, PV-2 or VSV. In each case, the calculated m.o.i. was adjusted to 1. After adsorption for 90 min at 32 °C, the inoculum was removed, the cells were washed twice, re-fed with 10 ml of maintenance medium and incubated at 32 °C. Cell cultures were harvested either when c.p.e. was complete or after 5 days of incubation if the amount of c.p.e. was minimal; these fluids were clarified at 400 g and assayed for infectivity. The viral yields from HRV-1 and PV-2 superinfected cultures were typed with appropriate specific antisera whereas VSV was distinguished by its characteristic c.p.e. and high yields of infectious virus. Supernatants were assayed for viral yields and these results were compared to those obtained in normal HeLa cells.

Indirect immunofluorescence. Persistently infected HeLa cells were grown on coverslips or four-well chamber slides (Lab-Tek). Guinea-pig antiserum with a neutralizing titre for HRV-2 of 10^4/100 TCID₅₀ was diluted 1:20 and added to the cells. Controls consisted of uninfected HeLa cells and PI cells treated with either phosphate-buffered saline (PBS) or pre-immune guinea-pig serum, and RV-2-infected HeLa cells treated with either immune or pre-immune guinea-pig serum. The cells were fixed in acetone at −20 °C for 10 min, incubated with antisera at 37 °C for 1 h and washed three times with PBS. Fluorescein-conjugated anti-guinea-pig IgG (Miles Laboratories) was added to the cells. After incubation at 37 °C for 30 min, the coverslips were washed twice with PBS, dried on filter paper, mounted onto a slide under buffered glycerol and examined under a Zeiss universal scope with epifluorescent illumination.

Interferon assay. The supernatant fluids from persistently infected cells were adjusted to pH 2.0 with 1 M-HCl and incubated for 30 min at room temperature to inactivate any HRV present. The pH was then raised to 7.5 with 1 M-NaOH and 3 ml of twofold serial dilutions were added to duplicate HeLa cell cultures which were incubated at 37 °C for 18 to 24 h. After aspiration of fluids, treated cultures were challenged with 100 p.f.u. of VSV. Human fibroblast reference interferon from the Reference Reagents Branch of the National Institute of Allergy and Infectious Diseases was diluted to contain 4 units of interferon and used to verify the sensitivity of the test. Interferon titres are expressed as the reciprocal of the highest dilution of test material resulting in a 50% reduction in challenge virus plaques.

Viral RNA synthesis. HeLa cells were grown in 24-well tissue culture dishes (Costar). At time 0, cells were infected with virus at an approximate m.o.i. of 8 to 12 followed by adsorption at 32 °C for 30 min. The inoculum was removed, and the monolayers were washed three times with maintenance medium. Maintenance medium containing actinomycin D (3 μg/ml) (Sigma) was added to all wells except controls. Following a 30 min incubation at 32 °C, 5 μCi of ³H-labelled uridine (ICN) (sp. act. 33 Ci/mmol) was added to each well and the plates were incubated at 32 °C. At predetermined time intervals, the medium was removed, monolayers were washed twice with cold Earle's balanced salt solution containing 0.2% FBS and twice with PBS and then cells were solubilized with 1% SDS in sodium acetate buffer (0.01 M, pH 5). Acid-insoluble radioactivity was precipitated onto Whatman 3MM filter paper discs by three washes with 10% TCA followed by two absolute ethanol washes. The discs were dried, put into toluene-based scintillation cocktail and counted in a Beckman LS 7000 scintillation spectrophotometer.

RESULTS

Establishment of persistent infection

HeLa cells were grown to confluence in 3 oz prescription bottles and infected in duplicate or quadruplicate with different m.o.i.s of either the ts⁻ mutants or HRV-2. Cultures were incubated at 32 °C or 37 °C and observed daily for c.p.e. All cultures incubated at 32 °C were destroyed by viral replication within 72 h. At 37 °C, all cultures infected with HRV-2 were also destroyed and repeated attempts to establish carrier cultures were unsuccessful. In contrast, cultures infected with ts⁻ mutants and incubated at 37 °C showed various degrees of c.p.e. ranging from barely detectable to involvement of over 90% of cell monolayers. Where necessary, cultures were re-fed frequently until a stable and cultivable cell population was formed. As seen in Table 1, all the mutants were capable of establishing PIs in HeLa cells, but initiation was somewhat dependent on the m.o.i. The lowest m.o.i. tested was 0.0001, and four out of five mutants did not establish persistent infections at this multiplicity. On the other hand, all mutants at a m.o.i. of 0.001, and three mutants at an m.o.i. of 0.01 established stable PIs. Unless otherwise noted, the results presented in the remainder of the report were obtained with the TS-1-initiated PI system.
Table 1. **Effect of m.o.i. on establishment of persistent infection in HeLa cells at 37 °C with five ts mutants**

<table>
<thead>
<tr>
<th>Virus</th>
<th>0.01</th>
<th>0.001</th>
<th>0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS-1</td>
<td>+*</td>
<td>+</td>
<td>−†</td>
</tr>
<tr>
<td>TS-2</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>TS-3</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>TS-4</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>TS-5</td>
<td>‡</td>
<td>‡</td>
<td>‡</td>
</tr>
<tr>
<td>HRV-2</td>
<td>‡</td>
<td>‡</td>
<td>‡</td>
</tr>
</tbody>
</table>

* Persistent infection established.
† No infectious virus recovered from these cultures during six subcultures.
‡ Cultures were destroyed by virus 2 to 4 days after infection and could not be rescued.

**Properties of the PI system**

Infectious virus was detected in all supernatants tested, except in those cultures which cured spontaneously or were cured with specific antiserum (see below). Fig. 1 shows the results of plaque assays of extracellular virus at various time intervals after initiation of PI. The amount of infectious virus found in the extracellular fluids generally ranged from 10^3 to 10^4 p.f.u./ml. At six intervals between weeks 21 and 44, the percentage of cells producing infectious centres varied from 0.03% to 23% (data not shown). All PI cultures went through unpredictable cyclical phases of crisis and recovery. Crisis periods were usually characterized by a destruction of ≥90% of the monolayer. The occurrence and duration of the crisis were unpredictable, lasting from several days to as long as 8 to 9 weeks. During this time, cultures were re-fed frequently until the cells formed a monolayer and stability was achieved.

**Properties of virus recovered from PI cultures**

Virus recovered from PI cultures (HRV-PI) possessed characteristics that were different from the parental virus of the PI. Efficiency of plating (32 °C/37 °C) values ranging from 0.32 to 1.17 obtained for the persistent virus isolated from subcultures 2, 4, 5, 6, 7, 9 and 29, indicated that the virus was no longer temperature-sensitive when compared to a value of 0.001 for the initiating virus. Also, a shift-down of the PI culture to 32 °C did not result in an increase in c. p.e. indicating that the virus isolated from the PI was phenotypically wild-type (ts+) for temperature...
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Table 2. Viral susceptibility of PI* and uninfected HeLa cells

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>Yield (p.f.u./ml) PI cells/uninfected cells</th>
<th>Interference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRV-2</td>
<td>$4.9 \times 10^4/1.0\times 10^6$</td>
<td>95.1</td>
</tr>
<tr>
<td>TS-1</td>
<td>$1.2 \times 10^3/1.1\times 10^6$</td>
<td>99.0</td>
</tr>
<tr>
<td>HRV-4</td>
<td>$5.8 \times 10^2/5.0\times 10^6$</td>
<td>88.4</td>
</tr>
<tr>
<td>PV-2</td>
<td>$1.8 \times 10^8/1.3\times 10^8$</td>
<td>Ns†</td>
</tr>
<tr>
<td>VSV</td>
<td>$2.1 \times 10^8/2.8\times 10^8$</td>
<td>Ns</td>
</tr>
</tbody>
</table>

* Mock-superinfected cultures produced $9.3 \times 10^2$ p.f.u./ml of culture supernatant.
† Ns, Not significant.

Table 3. Susceptibility of spontaneously cured PI cells to superinfection after subculturing

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>Yield (p.f.u./ml) cured cells/uninfected cells: number of subcultures</th>
<th>Interference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRV-2</td>
<td>$2.5 \times 10^4/1.0\times 10^7 (99.8)^* $</td>
<td>$2.0 \times 10^4/1.3\times 10^6 (84.6)$</td>
</tr>
<tr>
<td>TS-1</td>
<td>$7.9 \times 10^3/1.0\times 10^7 (99.9)$</td>
<td>$1.0 \times 10^5/2.0\times 10^6 (95.0)$</td>
</tr>
<tr>
<td>HRV-4</td>
<td>$1.6 \times 10^6/1.6\times 10^6 (None)$</td>
<td>ND†</td>
</tr>
</tbody>
</table>

* % Interference.
† ND, Not done.

sensitivity. When tested with hyperimmune TS-1 serum, the neutralizing titres obtained with TS-1 virus and virus obtained from several PI subcultures were identical (data not shown).

Curing of PI cultures

Cell cultures from the 40th subculture of the TS-1 PI system were subcultivated in quadruplicate and half the cultures were grown in the presence of 100 units of TS-1 antiserum. Three serial subcultivations in the presence of antiserum resulted in a cure (no extracellular virus or infectious centres could be detected). In addition, no infectious virus could be detected after 12 further subcultures in the absence of antiserum. During routine subcultivation of PI cultures, some spontaneous cures also occurred. No viral antigens could be detected by immunofluorescence in either spontaneously cured cultures or in those cured with antiserum.

Superinfection

Homologous and heterologous viruses at m.o.i.s of 1:0 were used for superinfection experiments. PV-2, VSV, HRV-4, HRV-2 or TS-1 were inoculated into duplicate bottle cultures of uninfected HeLa cells and PI cells. The cultures were examined daily for c.p.e. for 5 days and compared to PI cells which were not superinfected. The c.p.e. observed in cells superinfected with HRV-2, TS-1, and HRV-4 were similar to those produced in PI cells alone. In contrast, VSV- and PV-2-superinfected cultures were completely destroyed 24 to 36 h post-infection. The viral yields from superinfected PI cells were compared to those obtained in normal HeLa cells infected with the same viruses. Progeny virions from HRV-4- and PV-2-superinfected PI cultures were identified with appropriate specific antisera. There was essentially complete interference with the replication of TS-1, 95% interference with the replication of HRV-2 and significantly less ($P < 0.05$) interference with the heterologous HRV-4 (Table 2). PV-2 and VSV yields were not affected.

The sensitivity of spontaneously cured (Table 3) and antiserum-cured (Table 4) cells to superinfection was also examined. The results indicate that after cell cultures are cured and routinely subcultured (weekly, 1:4 split ratios), the sensitivity to superinfection increases. This phenomenon appears to occur earlier in antiserum-cured cells than in cultures that cured spontaneously. In addition, cured cells regain sensitivity to the HRV-4 heterologous virus after only one or two subcultivations.
Table 4. **Susceptibility of antiserum-cured cells to superinfection after subculturing**

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>Yield (p.f.u./ml) cured cells/uninfected cells:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of subcultures</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>13</td>
</tr>
<tr>
<td>HRV-2</td>
<td>$2.0 \times 10^7/1.0 \times 10^6$ (80.0)*</td>
</tr>
<tr>
<td>TS-1</td>
<td>$1.6 \times 10^6/1.0 \times 10^6$ (98.4)</td>
</tr>
<tr>
<td>HRV-4</td>
<td>$6.3 \times 10^6/6.0 \times 10^6$ (None)</td>
</tr>
<tr>
<td></td>
<td>$5.0 \times 10^6/1.0 \times 10^7$ (50.0)</td>
</tr>
<tr>
<td></td>
<td>$1.0 \times 10^6/1.0 \times 10^7$ (90.0)</td>
</tr>
<tr>
<td></td>
<td>ND†</td>
</tr>
<tr>
<td></td>
<td>$7.9 \times 10^5/1.3 \times 10^6$ (37.0)</td>
</tr>
<tr>
<td></td>
<td>$7.9 \times 10^5/2.0 \times 10^6$ (60.5)</td>
</tr>
</tbody>
</table>

* % Interference.
† ND, Not done.

**Interferon**

We examined supernatant fluids from PI cultures for interferon activity. The sensitivity of our tests was monitored by including 4 units of reference interferon treated in the same manner as the fluids from PI cultures. Fluids examined for interferon activity were not frozen prior to testing. The supernatants from two different passages of PI cultures (25 and 42) and a supernatant from a subculture of antibody-cured PI cells were tested for interferon. No interferon activity was demonstrated in any of the supernatants. No loss in reference interferon titres occurred during these tests.

**Viral RNA synthesis**

We studied the accumulation of virus-specific RNA in HeLa cell cultures infected with either HRV-PI, TS-1 or HRV-2 virus at both 32 °C and 37 °C. This was done by following incorporation of $^3$H-labelled uridine into viral nucleic acid. The calculated input multiplicities were the same for all virus preparations tested. Fig. 2 shows that at 32 °C significantly less labelled uridine was incorporated into the RNA of HRV-PI than into the RNA of either TS-1 or HRV-2. However, when viral infectivity yields from these experiments were quantified 24 h post-infection at 32 °C, no differences could be detected between yields of HRV-2, TS-1, and the HRV-PI viruses. Although not shown, the kinetics of RNA synthesis at 37 °C were similar to results obtained at 32 °C.

**DISCUSSION**

Persistent infections in vitro are characterized either by the continuous or intermittent release of infectious virus or by the production of viral antigens by cultured cells which have survived infection by a normally lytic virus. We have successfully established a PI of HeLa cells with a ts-mutant of HRV-2 at the restrictive temperature and have maintained the system for 45 passages over a period of 18 months. The virus isolated from the PI was shown to be of a ts$^+$ phenotype, indicating that temperature sensitivity was not essential for the maintenance of the system. More frequently, rapid selection and accumulation of ts$^-$ mutants is observed (Williams et al., 1981; Preble & Youngner, 1972; Hodes, 1982). In each of these cases, however, either a subpopulation or a variant of the original input virus population was selected. It has been shown by Holland et al. (1979) that multiple viral genome mutations evolve during long-term PI by VSV and that the resultant mutants are phenotypically and genotypically different from the initiating virus. Thus, the ts$^+$ phenotype of our PI virus is probably not due to a reversion back to wild-type but is more likely a variant subpopulation of virus present in the parental stock. In accordance with this, recent studies done in this laboratory indicate that persistent infections also can be initiated in HeLa cells by infection with HRV-2 (wt) virus when appropriately lower m.o.i.s are used.

Takemoto & Habel (1959) reported that the persisting or derived virus in their system could be differentiated from the original Coxsackie A9 virus by neutralization tests using polyclonal antiserum. We have not been able to show a difference between our initiating virus and the PI-derived virus with respect to serological type by this method. HRV-PI achieves viral yields...
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Fig. 2. Accumulation of virus-specific RNA in HeLa cells infected in the presence of actinomycin D (3 µg/ml). ●●●●, HRV-2; ●●●●, TS-1; ●●●●, HRV-PI. Bars represent the standard error of the mean.

similar to those of HRV-2 and TS-1 upon infection of HeLa cells but is able to do so with less total RNA synthesis. This may result either from more efficient packaging or from a greater ratio of functional to non-functional genomic RNA being packaged.

Interference with the replication of homologous rhinovirus in the superinfection experiments may be due to the absence or blocking of host cell receptor sites or possibly to inhibition of an intracellular event vital to replication of the virus. Once the PI cells are cured, there is a decrease in inhibition of homologous virus. Further, viral antigens cannot be detected by indirect immunofluorescence. Such interference is compatible with the presence of genetically resistant cells which have been reported for other PI systems (Ahmed et al., 1981). However, the return to susceptibility of cured cells suggests that permissive cells present in the rhinovirus PI system become prevalent once the virus is removed. Antiserum-cured cells regained sensitivity earlier in passage than spontaneously cured cells. This may be due to the presence of a greater population of permissive cells in such cultures, since curing with antiserum probably occurs when the number of permissive cells in the culture is still relatively high and less time is required for their recovery. It appears that in our PI system, a selective process is occurring, resulting in a dynamic population of both permissive and non-permissive cells and when the number of permissive cells becomes too small to support continued replication, spontaneous cures may occur.

Although DI particles have been produced by other picornaviruses (McClure et al., 1980; Radloff & Young, 1983), their presence in populations of rhinoviruses has not been documented. The interference demonstrated by our superinfection experiments was consistent with the presence of DI particles, but we were unable to demonstrate interference indicative of DI
particles by coinfection experiments using a method similar to that reported by Andzhaparidze et al. (1982). The existence of 'dense particles' which exhibit a lower specific infectivity has been reported in populations of picornaviruses (Rowlands et al., 1975; Yamaguchi-Koll et al., 1975) including rhinovirus (Gauntt et al., 1975). Although the presence of similar particles in our PI systems is possible, it is unlikely that these would play any role in maintenance since their presence does not appear to interfere with replication (Gauntt et al., 1975).

The inability to detect interferon in our system does not conclusively exclude its presence; however, the absence of interference with heterologous virus replication (PV-2, VSV) suggests that this is the case. As demonstrated by Ramseur & Friedman (1978) and Sekellick & Marcus (1979), a possibly more sensitive method of detecting interferon activity would be the use of anti-interferon antibody in the PI culture. Since rhinoviruses are known to be sensitive to interferon (Came et al., 1976), additional attempts to demonstrate the presence of interferon in our PI system would be warranted.

Thus we have been unable to implicate the most commonly reported mechanisms in the modulation of our PI system. The selection of a cell population resistant to virus infection appears to be the primary means by which the culture eludes destruction. The ability of the virus to persist and cause crisis may be due to its adaptation to these newly selected cells or simply to the presence and outgrowth of permissive cells. Additional studies are needed to characterize these aspects of the virus–host cell interaction.

REFERENCES


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