MRC-5 Cells, a Model for Junín Virus Persistent Infection

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SUMMARY

Persistent infection of MRC-5 cells was established following inoculation with attenuated Junín virus (JV). In the acute phase of the infection both the pathogenic XJ and the attenuated XJ0 and XJC13 strains showed severe c.p.e. and free viral titres reached $10^5$ p.f.u./ml. Recovery and establishment of persistently infected MRC-5 sublines (MRC-5p) proved a very common event and seemed to be independent of viral strain, m.o.i. employed or virus passage history. These MRC-5p sublines released virus throughout their life span and infectious centre assays performed at different passage levels with two sublines showed that 5 to 9% of the cells were producing virus. Heterotypic but not heterologous resistance to superinfection developed, as observed in persistent JV–heteroploid cell systems. Analysis of released JV showed that attenuation had not been markedly altered, but alteration in plaque morphology under methyl cellulose, appearance of temperature-sensitive mutants and alterations in mouse pathology imply that some properties of JV have been altered. Results presented here stress once again the ability of JV to establish persistent infections. The source and diploid characteristics of MRC-5 cells make them a satisfactory model for JV persistence studies.

During the past few years the study of persistent viral infection has become a focus of increasing attention following recognition of its frequency and clinical significance. Persistence in the animal host as a whole, although involving a complex interplay of various factors including viral tissue tropism and the host’s immune response, ultimately depends on persistence at the cellular level. In this regard, the study of the fundamental mechanisms of this particular virus–cell relationship must be carried out in simpler experimental models, such as cell culture systems.

Junín virus (JV), aetiological agent of Argentine haemorrhagic fever (AHF) and member of the Arenaviridae family, is able to induce both persistent and acute lytic infection in several hosts (Weissenbacher & Damonte, 1983). This effect has been demonstrated in several experimental models either in vitro, as in Vero (Boxaca, 1970; Boxaca et al., 1972; Boxaca & Giovanniiello, 1977) and BHK-21 cells (Carballal et al., 1980), or in vivo, mainly in guinea-pigs (Malumbres et al., 1984), mice (Sabattini et al., 1977; Lampuri et al., 1982) and rats (Laguens et al., 1983).

As the risk of JV persistence in human infection with wild-type virus or attenuated strains used as vaccines cannot be ruled out, the development of an in vitro experimental model is of considerable interest. Although JV persistent infection in continuous cell lines has already been described, human diploid cells, taking into account their origin, greater homogeneity and closer resemblance to in vivo tissues, would appear to provide a better understanding of events taking place at the host cellular level.

We have chosen MRC-5 cells as a model for studying the possible alterations induced in cell physiology by JV persistent infection, which may result in dysfunction of otherwise normal cells as has been shown for lymphocytic choriomeningitis virus in an in vitro model (Oldstone et al., 1982).
Here, we describe the ready establishment of persistent infection of MRC-5 human diploid cells, and partial characterization of sublines persistently infected with an attenuated strain of JV.

The infection of MRC-5 cells with the pathogenic XJ or attenuated XJC13 or XJ0 strains of JV developed in two stages: the acute phase involved active viral replication and c.p.e. followed by cell recovery leading to a chronic phase which lasted throughout the culture life.

Although the three growth curves showed a similar trend during the acute phase, titres for XJ0 were uniformly and significantly higher than for XJ or XJC13. C.p.e. appeared earlier in XJ- or XJC13-infected cultures, starting with localized foci of rounded, dark, necrotic cells, which progressed until almost complete monolayer destruction. For XJ0, c.p.e. appeared later, at day 8 post-infection, characterized by a widespread alteration of the monolayer. After adopting a spindle shape most cells detached from the plastic surface, while the remaining ones presented a transient bizarre morphology. Cell growth became evident at 12 days post-infection for all three strains, replenishing the monolayer in a few days.

In this phase viral titres were similar to those in heteroploid cells (Vero, BHK-21), but significantly higher than in WI-38, the other human diploid strain studied (our unpublished data), showing that the MRC-5 strain, an accepted substrate for human vaccine production, is satisfactory for JV growth. Differences were minimal among the three virus strains studied. The higher XJ0 titres could be ascribed to the greater m.o.i. employed. Although differences in c.p.e. were not significant enough to identify this strain they may indicate differences in the viral growth mechanism. Furthermore, at least for XJ0, initiation of c.p.e. seemed to be unrelated to m.o.i.

Seven cultures of MRC-5 cells were inoculated with different XJ0 stocks and doses as described in Table 1, and incubated at 37 °C. Eagle's basal medium with 10% and 2.5% foetal bovine serum was used for growth and maintenance respectively. The infected cultures and parallel uninfected controls were maintained in similar conditions, and subcultured when the MRC-5-infected cells (MRC-5m) reached confluence. Cells were counted in the seeding suspension and when subculturing to determine the number of duplications. Supernatants harvested at various times after infection were fractioned and kept at −70 °C after centrifuging for 10 min at 1000 r.p.m.

The seven infected MRC-5 cultures exhibited the described c.p.e. between 6 and 24 (median 8 to 13) days post-infection followed by cell growth that lasted from days 12 to 40 post-infection (median 16-5 to 19-5 days) (Table 1). Two out of these seven sublines, namely S1 and S2, were lost
Table 2. Characteristics of JV released by MRC-5_p1 substrains 1 and 6*

<table>
<thead>
<tr>
<th>MRC-5 subline</th>
<th>Subculture (days post-infection)</th>
<th>Plaque morphology†</th>
<th>Viral titres (p.f.u./ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>37 °C</td>
<td>39 °C</td>
</tr>
<tr>
<td>1</td>
<td>0 (parental virus)</td>
<td>Lytic</td>
<td>1.2 x 10⁷</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Bull’s eye</td>
<td>1.1 x 10³</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Turbid, small</td>
<td>3.6 x 10⁴</td>
</tr>
<tr>
<td>6</td>
<td>0 (parental virus)</td>
<td>Lytic</td>
<td>2.85 x 10⁴</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Bull’s eye</td>
<td>2.0 x 10⁵</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Lytic</td>
<td>2.5 x 10⁵</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Lytic</td>
<td>5.1 x 10⁴</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Turbid +</td>
<td>2.8 x 10³</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Turbid +</td>
<td>5.1 x 10³</td>
</tr>
</tbody>
</table>

* Fluids from MRC-5_p1 cultures were grown in Vero cells at 37 °C and then titrated by the plaque-forming technique under methyl cellulose at 37 °C.
† Observed by the technique mentioned above.

due to bacterial contamination, the former at the end of the recovery phase (13 days post-infection) and the latter at 70 days post-infection. In spite of rather abnormal morphology and slower growth, the MRC-5_p1 cells that replenished the monolayer reverted to normal appearance and growth after the first subculture, and became indistinguishable from controls. In both MRC-5_p1 and MRC-5 normal cells the cell population doubled at each subculture. After full recovery, the life span of three out of the five sublines paralleled their respective controls, while S₁ and S₃ died 25 to 30 days before their controls.

All MRC-5_p1 sublines were found to produce virus throughout the life span of the cells. In S₁, virus production followed an alternating pattern with differences in successive titres being between 1 and 2 log₁₀ p.f.u., titres ranging from 1.1 x 10⁵ (33 days post-infection) to 6.5 x 10⁵ p.f.u./ml (70 days post-infection).

In contrast, S₆ produced virus more steadily, with titre differences less than 1 log₁₀ p.f.u. up to 85 days post-infection when a marked drop of 3 log₁₀ p.f.u. preceded culture death at 128 days post-infection. Titres oscillated between 2.8 x 10³ and 6.6 x 10⁵ p.f.u./ml. Infectious centre assay performed at 35 days (S₆) and at 52 days (S₄) post-infection showed that 5 to 9% of the cells were producing virus.

Superinfection with 10³ p.f.u. of the homologous XJC13 or with the antigenically related Tacaribe virus failed to induce c.p.e. in MRC-5_p1 cells, whereas a rapid destruction of the culture was seen with 10⁵ p.f.u. of the heterologous virus vesicular stomatitis virus. In both cases, simultaneously infected MRC-5 controls were severely affected.

Under stringent experimental conditions establishment of persistent infection, which regularly followed culture recovery, proved a very common event and seemed to be independent of the viral strain, m.o.i. employed, and of the virus passage history, thus resembling the behaviour of JV and other arenaviruses in heteroploid lines (Boxaca, 1970; Staneck et al., 1972; Damonte et al., 1981, 1983).

In other virus-cell systems it has been demonstrated that host-cell metabolism and membrane structural composition can be altered by this type of viral infection (Parry et al., 1979; Anderton et al., 1982). Although MRC-5_p1 cells looked quite healthy, further study is necessary to determine whether persistent JV exerts a similar effect and to what extent it may alter normal cell function.
Continuous virus release is not the rule in JV or other Vero–arenavirus persistent cultures and when found, as with Tamiami and Pichinde viruses (Damonte et al., 1983), it was ascribed to the higher percentage of cells containing viral antigen as compared to the Vero–Junin system, where free virus could seldom be detected (Boxaca, 1970; Coto et al., 1977). In our case the infectious centre assay showed values which correlated with the viral titres found. This absence of typical c.p.e. indicates that resistance to homologous superinfection is also developed in the MRC-5pI cells. However, in arenavirus–heteroploid systems this lack of c.p.e. does not necessarily rule out superinfecting virus replication, as antigenic relationships between persistent and superinfecting virus have been advanced to account for this carrier-culture property (Boxaca et al., 1972; Damonte et al., 1983). Whether this is the case in our system remains to be seen.

Although viral isolates from MRC-5pI cultures were identified as JV by a neutralization test with anti-JV serum, some differences from the parental strain were detected. Plaque morphology under methyl cellulose was irregular, ‘bull’s eye’, turbid or lytic (standard) plaques being observed in a random sequence, and the 39/37 °C titre ratio suggested the appearance and selection of temperature-sensitive (ts) mutants (Table 2). Like the parental XJ0, isolates from S1 or $S_0$ inoculated in Vero cells at different m.o.i., after an acute phase with marked c.p.e. production, started new persistent Vero sublines which proved resistant to homotypic but not to heterologous superinfection.

Virulence of persistent JV was tested in 2-day-old Wistar rats and guinea-pigs. The intracerebral (i.c.) inoculation of $S_1$ fluids harvested at 56, 70 and 120 days post-infection in groups of 10 rats each caused 60 to 100% mortality in each case, whereas no deaths were observed when similar groups of rats were infected by the intraperitoneal route. Guinea-pigs infected with 100 p.f.u. of $S_1$ persistent virus isolated after 100 days post-infection showed low mortality, with only four out of 14 animals dying (28%). As expected, the survivors had specific JV-neutralizing antibodies and were protected against challenge with the pathogenic XJ strain of JV.

Although i.c. mouse inoculations of $S_1$ supernatants regularly killed the animals, pathological signs were rather different from the classic XJ encephalitis (Rabinovich et al., 1983). Tremors and gait lateralization were obvious but there was no final hind limb paralysis characteristic of pathogenic XJ strain. Also, the temporal distribution of deaths was different and is currently under study.

The appearance of ts mutants and defective interfering (DI) particle production has been proposed as mechanisms for JV persistent infection control in some heteroploid cells (Coto et al., 1977). In the MRC-5 diploid cell model, although the selection of ts mutants was also observed suggesting their role in maintaining JV persistence, the possibility of DI particles as another regulatory factor is still an open question.

Analysis of persistent JV isolated from MRC-5pI showed that attenuation had not been markedly altered as confirmed in both the guinea-pig and the rat, the two animals used. On the other hand, alterations in plaque morphology and ts characteristics, together with changes in mouse pathogenesis, imply that the JV genome has undergone certain changes which depend on host and/or virus–cell relationships for expression.

The results presented here again stress the ability of JV to establish persistent infections. The human origin and diploid characteristics of MRC-5 cells suggest that persistence cannot be ruled out in the human host.

Although no clinical evidence suggested this type of persistence in a limited trial of 636 volunteers immunized with the XJC13 strain, a strain closely related to XJ0 (Ruggiero et al., 1980), and followed up to 10 years later, the prospect of preventative vaccination against AHF with an attenuated virus obviously requires deeper study of JV persistence.

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Short communication

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