Cyclic Expression of Antigen and Infectious Virus in a BHK Cell Line (0-853) Persistently Infected with an SSPE Strain of Measles Virus

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SUMMARY

Establishment and characteristics of a baby hamster kidney cell line (BHK 0-853) persistently infected with a subacute sclerosing panencephalitis (SSPE) strain of measles virus (Lec strain) is described. The persistent infection was easily and repeatedly established and no special conditions were required. There was a predictable fluctuation in expression of virus intracellular and membrane antigens which varied from greater than 90% to less than 1% of the cells demonstrating these antigens during the first 6 or 7 passages. Thereafter, fluctuation of antigen and infectious virus expression continued in an unpredictable fashion.

The ability of a subacute sclerosing panencephalitis (SSPE) strain (Lec) of measles virus (Barbanti-Brodano et al. 1970) to establish a chronic infection was studied in various cell lines (two African green monkey cell lines, Vero and CV-1; two transformed baby hamster kidney cell lines, BHK21 and 0-853; and a human foetal diploid line, HFDL 645, established in our laboratory). Cells were infected in suspension with 0.01 p.f.u./cell for 1 h at 36 °C. The medium was Eagle's medium in Earle's salts supplemented with 10% foetal bovine serum, 10 mM-NaHCO$_3$, 2 mM-glutamine and antibiotics. The virus was at passage (P) 18 (P17 in CV-1 and P1 in Vero cell lines). With the exception of the BHK 0-853 cell line, infection of the other lines resulted in complete destruction of the monolayers within one passage (in CV-1, Vero and HFDL cell lines) or within two or three passages (BHK21 cell line). These infected cultures could not be passed. No c.p.e. was seen in infected BHK 0-853 cells until P2 and P3, and the infected cells initially could be passed at a 1:2 to 1:3 split and, like the non-infected cells, could be subsequently passed at a 1:20 to 1:25 split.

The infected cell line was followed for one year for membrane and intracellular staining for measles antigens by the fluorescent antibody technique (FA) using fluorescein-conjugated gamma globulin from sera of hamsters immunized to measles virus HNT, Philadelphia strain 26, adapted to hamsters (Burnstein et al. 1964). Less than 1% of the viable cells showed membrane staining from day 1 to day 7 after the primary infection (P0). At P1 the percentage of staining cells increased with days of culture and by day 4 usually 30 to 50% of the cells had membrane staining. At P2 and P3, 80 to 100% of the cells showed membrane staining. At P4 the percentage of such cells dropped and by P5 and P6 was less than 1%. Expression of intracellular antigen followed the same temporal pattern as membrane antigen except that it was expressed in approx. 100% of the cells for one passage longer (i.e. to P4) before dropping. By P6, less than 1% of the cells had intracellular stain. Thereafter, expression of both intracellular and membrane antigens fluctuated in an unpredictable fashion (Fig. 1).

The yield of intracellular and extracellular infectious virus, determined by plaque assay, also fluctuated as much as 1000-fold between some passages and not always in complete synchrony with the fluctuation in FA. Analyses by sucrose gradient centrifugation of
Fig. 1. Fluctuation of membrane (closed circles) and intracellular staining (open circles) for measles antigen in persistently infected BHK 0-853 cells over a one year period. Staining was checked at 72 to 96 h after passage of the cells (a) and/or at 48 h (b). Solid line = staining at 72 to 96 h; broken line = staining at 48 h.

infectious virus from concentrated culture fluids at P2 and P5 indicated that the recovered virus banded at the same density (1.18 to 1.19 g/ml) as virus from a lytic infection in the monkey cell lines. The majority of plaques were red plaques less than 1 mm in diam. which became clear after an additional 24 h of incubation.

At passages with a high percentage of staining cells, virions and smooth and granular nucleocapsids were seen by thin section electron microscopy. Smooth nucleocapsids were in the nucleus and occasionally in the cytoplasm, while granular nucleocapsids were only in the cytoplasm.

Chronically infected cell lines could be initiated repeatedly and the same initial pattern of virus expression occurred as described above. If the multiplicity of infection (m.o.i.) was increased, the time for antigen expression in 80 to 100% of the cells was shortened by one passage (Table 1), but chronically infected cultures became established with equal ease. No special methods were required for their establishment, such as growth of infected cells in the presence of specific antiserum, use of undiluted virus preparations, or the re-feeding without passage of the few surviving cells in infected cultures. (Rustigian, 1966a, b; Norrby, 1967; Gould & Almeida, 1977; Minagawa, 1971; Chiarini et al. 1976; Ter Meulen & Martin, 1976; Rima et al. 1977).

The state of persistency of virus infection in culture is usually virus rather than host dependent and is associated with temperature sensitive mutants (Preble & Youngner, 1975) or with defective interfering particles (Huang, 1973). The ease with which chronically infected cultures could be repeatedly established with the BHK 0-853 cell line also suggests a contribution by the cell line in addition to that of the virus in the establishment of the persistent infection, a finding also noted by Wild & Dugre, 1978.
Table 1. Effect of m.o.i. on the production of membrane fluorescence

<table>
<thead>
<tr>
<th>M.o.i.</th>
<th>P0†</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
</tr>
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<td>0·01</td>
<td>&lt; 1</td>
<td>10-50</td>
<td>80-100</td>
<td>70-100</td>
<td>10-40</td>
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<td>30-70</td>
<td>70-100</td>
<td>10-60</td>
<td>&lt; 1--&lt; 10</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>1·0</td>
<td>&lt; 1</td>
<td>100</td>
<td>80-100</td>
<td>10-60</td>
<td>&lt; 1--&lt; 10</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

* Range of results from four different experiments.
† Cells were infected in suspension and dispersed on to coverslips. This first set of coverslip cultures is designated P0.

The BHK o-853 cell line was established in 1966 by J. L. Riggs of this laboratory from hamsters of our colony, which was started with stock from the National Institutes of Health. The cell line formed encapsulated, poorly differentiated sarcomas upon subcutaneous inoculation into hamsters. At earlier passages (P102) the cell line was near diploid with 44 chromosomes and with approx. 18% polyploidy. At a passage (P419) at the time of this study, it was in the near tetraploid range, with 66 to 74 chromosomes. The cell line was periodically checked for mycoplasma under anaerobic and aerobic conditions and was negative. Hamster type A, type C and type R viruses have been seen periodically in this cell line by electron microscopy (Bernhard & Tournier, 1964; Compans et al. 1966). Although they were not detected during the period of observation of this study, the continuing presence of the genome of these viruses could exert an influence on the establishment of persistent infection with measles virus.

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REFERENCES
Short communications


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