Simian Virus 40 Infection Is Not Mediated by Lysosomal Activation

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(Accepted 18 May 1987)

SUMMARY
The uptake of simian virus 40 (SV40) virions to the nucleus, the site of viral replication, proceeds via engulfment at the cytoplasmic membrane and transport in monopinocytotic vesicles through the cytoplasm to the nuclear membrane. In the case of Semliki Forest virus and poliovirus which undergo primary endocytosis in a similar manner, neutralization of the acid pH in these vesicles abolishes viral infectivity. We have examined the effects of the lysosomotropic agents chloroquine and ammonium chloride on the uptake of SV40 and find that neutralization of the acid pH in cellular organelles has no effect on the progress of SV40 infection. Although the initial endocytotic pathway appears similar for the viruses, the vesicular transport of SV40 to the nucleus proceeds, therefore, via an alternative endocytotic compartment which is not inhibited by increasing the endosomal pH.

The infectivity of mammalian viruses requires the uptake and transport across the cytoplasmic membrane of the virus by the cell from the external milieu to an intracellular compartment. This usually requires the recognition by the virus of an extracellular receptor, membrane fusion, or vesicularization and delivery to the viral replicative site. Vesicularization appears to be a critical step in activation of the uncoating and infectivity process. A well documented example is Semliki Forest virus where transport through the acid compartment of intracellular vacuoles (adsorptive endocytosis) leading to lysosomes is essential for infectivity (Helenius et al., 1980): neutralization of the acid pH by chloroquine or NH₄Cl prevents activation of subsequent infectivity (Marsh et al., 1982, 1986; Kielian & Helenius, 1985; Mellman et al., 1986). Acidification of the endosome compartment is also necessary for entry of the non-enveloped polioviruses (Madshus et al., 1984a, b). In an analogous manner, simian virus 40 (SV40) virions attach to the surface of infectible cells, whereupon the virus is engulfed by budding of the cytoplasmic membrane and transported intracellularly (Dales, 1973). The rate of uptake of Semliki Forest virus and SV40 is similar also (Barbanti-Brodano et al., 1970; Hummeler et al., 1970; Helenius et al., 1980). In the case of SV40 the final destination is the nucleus where replication of the genome and maturation of the virus ensues although the vesicles have the capacity to fuse with any cellular organelle (Maul et al., 1978). This mechanism suggests activation of a specific transport function to provide access of the virus via the nuclear membrane to the nuclear environment itself. If the virus usurps a normal cellular function for this purpose (and it is difficult to accept that a virus as relatively simple as SV40 can do otherwise) such mechanisms have not been described previously. In this communication we have examined the effect of inhibition of endosome acidification by chloroquine and ammonium chloride on the infectivity of SV40.

Table 1 shows treatment of CV1P cells under various regimens with the same concentrations of ammonium chloride and chloroquine shown to be >99% inhibitory for Semliki Forest virus (Helenius et al., 1980), i.e. 10 mM-ammonium chloride and 0.1 mM-chloroquine. There is no detectable effect on the infectivity of SV40. In this experiment the agents were added to the cells 30 min before the addition of virus and remained with the cells during the infection. The same concentrations of ammonium chloride and chloroquine were also added 1 h prior to infection.
### Table 1. Effects of ammonium chloride and chloroquine on SV40 infectivity*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h) prior to addition of SV40 for 1 h</th>
<th>Time (h) left after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0.1 mM-Chloroquine</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>2. 10 mM-NH₄Cl</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>3. 0.1 mM-Chloroquine</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>4. 10 mM-NH₄Cl</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>5. 0.1 mM-Chloroquine</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>6. 10 mM-NH₄Cl</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>7. 1 mM-Chloroquine</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>8. 30 mM-NH₄Cl</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>9. 1 mM-Chloroquine</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>10. 30 mM-NH₄Cl</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>11. –</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Ammonium chloride or chloroquine were freshly dissolved in medium 199, and added to CV1P cells 30 min or 1 h prior to the addition of the SV40 at an m.o.i. of 2. The medium containing inhibitors remained during the 1 h virus infection and then was removed or left for a further 1 h as indicated. Cells were grown on 13 mm glass coverslips in 20 cm² plastic dishes, and were fixed 24 h post-infection when T antigen expression was maximal. The experiment was repeated three times; duplicate coverslips were counted for T antigen-positive cells (~2 × 10⁴ cells). The CV1P line of African green monkey kidney cells (Mertz & Berg, 1974) was propagated in medium 199 with 10% foetal calf serum. The small plaque strain 776 of SV40 (Sweet & Hilleman, 1960; Takemoto et al., 1968) was propagated (Ganem et al., 1976) at an m.o.i. of 0.001 and was plaque-purified (Mertz & Berg, 1974) through three cycles. T antigen (Pope & Rowe, 1964) was detected by indirect immunofluorescence using mouse anti-T serum, raised by injecting SV40-transformed BALB/c cells or nuclei from SV40-infected BALB/c cells 24 h post-infection into BALB/c mice, and fluoresceinated or peroxidase-conjugated rabbit anti-mouse serum as the second antibody. Cells were fixed for 24 h on coverslips at appropriate times in acetone at −20 °C. All experiments yielded 97 to 99% T antigen-positive cells by 24 h post-infection.

Again no effect was observed on the production of T antigen i.e. the progress of infectious virus to the nucleus and synthesis of early gene functions. Helenius et al. (1980) have shown that inhibition by chloroquine, the most efficient agent that they tested, was effective within the first 5 min of endocytosis and did not have an effect on the binding of the virus, endocytosis, or uptake of virus into vacuoles. Furthermore, concentrations of 0.05 mM were sufficient to reduce virus yields by 98%.

Table 1 shows that even at concentrations of 30 mM-ammonium chloride and 1 mM-chloroquine, 20-fold higher than those required for Semliki Forest virus, no inhibition of SV40 infectivity was detectable. These are the highest levels we could use without toxicity to the cells.

When chloroquine was added 30 min prior to infection, but removed when the virus was added, no reduction in Semliki Forest virus infection occurred (Helenius et al., 1980). A reduction of only 30% in infectivity was observed if chloroquine was added 1 h after the virus, but not before. Helenius et al. (1980) have concluded that inhibition occurred during the first 5 min of the infection process. Lines 5 and 6 of Table 1 demonstrate that when the ammonium chloride and chloroquine were added 30 min prior to infection, during the infection and 1 h post-infection there was no reduction in infectivity of SV40. When the highest concentrations were used for the same period, again no reduction was observed (lines 9 and 10).

Thus levels of chloroquine 20-fold higher than required for 98% inhibition of Semliki Forest virus infection and added 1 h before and for 2 h after the sensitive step of 5 min duration (Helenius et al., 1980), did not affect the SV40 infection process.

The effects of chloroquine and ammonium chloride are not a property of BHK21 cells alone since they have been observed with a number of viruses and cell types (for review, see Helenius et al., 1980) and in particular have been demonstrated to be active on CV-1 cells (Dales, 1973; P. Upcroft, unpublished observations). We have observed similar effects also with methylamine, another lysosomotropic agent (data not shown). These are the most potent lysosomotropic agents described and are very effective in the inhibition of Semliki Forest virus (Helenius et al., 1980). Neutralization of acidic vesicles also abolishes infectivity of the non-enveloped polioviruses (Madshus et al., 1984a, b).
Although Semliki Forest virus and SV40 are not of the same group of viruses, uptake is via adsorptive endocytosis in both cases and is well documented at high multiplicities of infection by electron microscopy (Maul et al., 1978; Helenius et al., 1980). One major difference in their infectious processes is that Semliki Forest virus replicates in the cytoplasm and SV40 proceeds to the nucleus for propagation. Both viruses are seen by electron microscopy to be associated with multiple intracellular vesicles in clusters and alone (Maul et al., 1978; Helenius et al., 1980). Disruption of the activity of intracellular organelles by raising the pH with lysosomotropic agents is not an effect of the agents on the virus, the binding of the virus to the cell, endocytosis or uptake of the virus into intracellular vacuoles: the infectious process is activated therefore by uptake of the viruses through an acid compartment in a cellular vesicle (Helenius et al., 1980). This observation clearly defines an endocytotic pathway through compartments that was thought originally to be aberrant and destroyed the virus particle (Dales, 1973; Lonberg-Holm & Philipson, 1974). Such a pathway does not appear to be necessary for infection by SV40. The primary endocytotic pathway remains similar, but inhibition of function by neutralization of the pH does not prevent SV40 infection. Therefore the vesicular transport of SV40 to the nucleus proceeds via an alternative mechanism, which has been delineated with the use of lysosomotropic agents.

The receptosome, an intermediate organelle of receptor-mediated endocytosis, as originally described, results from clustering of receptor-ligand complexes in coated pits on the cell surface. The ligand is then internalized into the non-coated intracellular receptosome ‘which selectively avoids fusion with the lysosomes and moves towards the Golgi region of the cell by saltatory motion’ (Willingham & Pastan, 1980). Receptosomes also have a polypeptide composition that is different from lysosomes, plasma membranes and other fractions (Dickson et al., 1983). Furthermore, the actual movement of lysosomes and hormone receptor-containing endosomes towards the perinuclear region has been dissected and has a different time scale (Herman & Albertini, 1983). An interesting possibility for SV40 transport to the nucleus would be a receptosome, or endosome (Mellman et al., 1986) whose primary function does not require acidification. Although the movement of this vesicle in the cell is towards the perinuclear region, its fate is not fusion with lysosomes, but delivery of its contents, e.g. hormones, to the nucleus.

I thank B. Carter for technical assistance. This research was supported by the National Health and Medical Research Council of Australia.

REFERENCES

Short communication


(Received 17 February 1987)