Establishment and Characterization of St Louis Encephalitis Virus Persistent Infections in Aedes and Culex Mosquito Cell Lines

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SUMMARY

Persistent infections with St Louis encephalitis (SLE) virus were established in three mosquito cell lines (Aedes albopictus, A. dorsalis and Culex tarsalis) and were maintained for over 2 years. All three persistently infected cell cultures shared two features: (i) no overt cytopathic effect and (ii) a relatively high proportion of cells infected (41 to 85%). The Aedes persistently infected cultures were resistant to superinfection with the homologous virus but not heterologous viruses. Two significant differences were observed between the Aedes and C. tarsalis persistently infected cell cultures: (i) viral titres in the A. albopictus and A. dorsalis cell cultures decreased slowly over time (the decrease was particularly marked in the A. albopictus cell cultures), whereas titres in the C. tarsalis cell cultures remained relatively constant and (ii) the addition of anti-SLE virus antibody led to decreased virus production in the C. tarsalis cell cultures (one of two cultures was cured of infection), whereas antibody had no effect on the persistently infected Aedes cell cultures. These results suggest that there may be significant differences in the regulation of viral replication and the maintenance of flavivirus persistent infections in mosquito cell lines of different origins.

INTRODUCTION

St Louis encephalitis (SLE) virus, a member of the family Flaviviridae, is an enveloped virus which contains a single-stranded positive sense RNA genome (Westaway et al., 1985). It is an arbovirus and is maintained in nature by a transmission cycle involving wild birds and Culex mosquitoes (Monath, 1980).

Although arboviruses replicate efficiently in both vertebrate and invertebrate cells, the outcome of viral replication in the two phyla can be very different. The usual result of infection of vertebrate cells in vitro is rapid cell death, whereas infected invertebrate cultures, although in some cases demonstrating transient or even severe c.p.e., readily evolve into persistently infected cultures (for reviews, see Stollar, 1980; Brown & Condreay, 1986).

Persistent infections of mosquito cells with flaviviruses have not been studied extensively and in most studies only the Aedes albopictus cell line has been examined. Flavivirus-infected mosquito cell cultures exhibit variable c.p.e. Persistently infected cultures produce small plaque (sp) and temperature-sensitive (ts) mutants and become resistant to superinfection with the homologous virus. Viral titres are usually very erratic and high titres may be detected for many months post-infection (Igarashi, 1979; Ng & Westaway, 1980; Kuno, 1982). There have been no reports indicating that flavivirus-infected mosquito cell cultures produce defective interfering particles or can be cured by antibody treatment although these characteristics have been observed in flavivirus-infected vertebrate cell cultures (Brinton, 1982; Schmaljohn & Blair, 1977; Katz & Goldblum, 1968).

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The present study was undertaken to characterize and compare persistent infections with a single flavivirus in several different mosquito cell lines. Persistent infections with SLE virus were established in cell cultures derived from tissues of *A. albopictus*, *A. dorsalis* and *Culex tarsalis* mosquitoes. Persistent infections were readily established and were maintained for over 2 years. Notably, the three persistently infected cell lines differed significantly with respect to virus yields and the effect of antibody treatment on virus production. Viral mutants were also generated and these are discussed in the following paper (Randolph & Hardy, 1988).

**METHODS**

*Viruses and antisera.* Stocks of SLE (LAV5-156) virus were made by passaging a portion of the original infected pool of *C. quinquefasciatus* mosquitoes (collected in Imperial County, California, U.S.A. in 1975) twice in the C6/36 clone of *A. albopictus* cells. Turlock (TUR) virus (a bunyavirus) and western equine encephalomyelitis (WEE) virus were isolated from mosquito pools collected in California. TUR virus was prepared as a 10% suckling mouse brain suspension in phosphate-buffered saline (PBS) pH 7.4. WEE virus was prepared from supernatant fluids of infected duck embryonic cultures. Stocks of SLE (Parton), Japanese encephalitis (JE) and yellow fever (YF) viruses prepared from infected mouse brain and the corresponding immune mouse ascites fluids (IMAF) were obtained from Dr R. Emmons (California State Department of Health Services). IMAFs to SLE, WEE and TUR viruses were prepared as described elsewhere (Sartorelli et al., 1966).

**Cells and media.** The three cell lines used to establish persistently infected cultures were *A. albopictus* (Singh, 1967) (obtained from American Type Culture Collection), *A. dorsalis* (Cahoon et al., 1978) and *C. tarsalis* (Chao & Ball, 1976). These cell lines were grown in Mitsushashi–Maramorosch (MM) medium supplemented with 20% foetal calf serum (FCS), 2 mm-L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin and 100 µg/ml gentamicin. The C6/36 cell line, originally derived from a clone of Singh's *A. albopictus* cells by Igarashi (1978), was maintained in modified Eagle's MEM (EMEM) supplemented with 5% FCS. All mosquito cells were grown at 28 °C and were passaged weekly at a 1:20 dilution in fresh medium. Vero and chick embryonic fibroblast (CEF) cultures were grown in EMEM containing 5% FCS at 36 °C in the presence of 5% CO₂. *A. albopictus*, *A. dorsalis*, *C. tarsalis* and Vero cell lines were tested for bacterial and mycoplasmal contamination using the Mycotrim-TC system (Hana Biologics, Berkeley, Ca., U.S.A.) incubated at 28 °C and 36 °C. No contamination was detected.

**Plaque assay.** Flaviviruses and WEE virus were assayed by plaque formation in Vero cells and TUR virus by plaque formation in CEF cells (Cahoon et al., 1979).

**Immunoperoxidase staining and focus assay.** The immunoperoxidase focus assay (IPFA) was a modification of that described by Volkman & Goldsmith (1982). Cell monolayers grown on 12-well Teflon-coated slides (Carlson Scientific, Peotone, Ill., U.S.A.) were inoculated with 10 µl of diluted virus and incubated for 1 to 1.5 h. Vero cell monolayers were then overlaid with EMEM containing 2% CM-cellulose and 2% FCS and were incubated at 36 °C. Mosquito cell monolayers were overlaid with MM medium containing 2% CM-cellulose and 10% FCS and were incubated at 28 °C. All slides were incubated for 3 days then washed in PBS pH 7.4 for 5 min, fixed in phosphate-buffered acetone–formalin for 2 min, washed again in PBS for 10 min and stored frozen at −70 °C or stained immediately. Infected foci were detected using an avidin–biotin complex (ABC) staining method (Hsu et al., 1981). Biotinylated horse anti-mouse IgG, biotinylated goat anti-rabbit IgG and ABC reagent were purchased from Vector Laboratories (Burlingame, Ca., U.S.A.); the staining procedure used was that suggested by the manufacturer. Viral titres were determined by counting immunoperoxidase-stained foci of infection and were reported as log₁₀ f.f.u./ml. Titres of wild-type (wt) virus determined by IPFA were consistently lower (approx. 0.5 log₁₀) than those determined by plaque assay.

**Quantification of viral antigen in infected cells.** Cells from infected cultures were resuspended in medium, allowed to attach to slides such that individual cells were well separated and then stained by the ABC method described above. The amount of stain deposited on 100 cells from each population was quantified with a scanning microdensitometer (model M85, Vickets Instruments) at a wavelength of 450 nm. The staining density of an individual cell was the value obtained for that cell minus the background density of an adjacent empty area. Cells with staining densities of 1 standard deviation (S.D.) above the mean staining density of a population of uninfected cells of the same cell type were considered virus-positive.

**Superinfection assays.** Duplicate tube cultures containing semi-confluent monolayers of persistently infected cultures were superinfected with SLE (LAV5-156), SLE (Parton), JE, YF, WEE or TUR virus at m.o.i.s of approximately 0.5, 0.4, 80, 0.08, 0.2 and 0.4 respectively. Supernatant fluids were collected from each duplicate culture at selected times after infection. Samples obtained on appropriate days from cultures of acutely infected mosquito cells and tubes in which virus was incubated in cell-free medium served as positive and negative controls respectively. Viral titres are reported as the mean of duplicate determinations. Plaques produced by JE and YF viruses could usually be distinguished from those produced by SLE virus in superinfected cultures based on differences in plaque morphology and time of appearance. To verify the identity of the virus assayed, titres were...
also determined after incubating samples with a 1:20 dilution of the appropriate neutralizing IMAF for 1 h at 36 °C. The viral titres determined by both methods were essentially the same. The titres reported in Table 2 were those determined in the absence of neutralizing antiserum, with the exception of YF virus in superinfected C. tarsalis Ctl cell cultures. In this case it was necessary to neutralize the SLE virus present in the samples in order to count the YF viral plaques.

RESULTS

Establishment of persistently infected cultures

Two cultures each of A. albopictus, A. dorsalis and C. tarsalis cells, designated Aa1 and Aa2, Ad1 and Ad2, and Ctl and Ct2 respectively, were infected with SLE (LAV5-156) virus at an m.o.i. of 150 p.f.u./cell. All of these cultures became persistently infected and were maintained for over 2 years. No c.p.e. was observed at any stage of infection, although a transient c.p.e. was seen in Ct2 cultures treated with virus-specific antibody (see below).

Viral titres in acutely and persistently infected mosquito cell cultures

Following infection, viral titres in A. albopictus, A. dorsalis and C. tarsalis cell cultures gradually increased, reaching a peak at 3 to 4 days post-infection (p.i.). Peak titres ranged from $10^{7.5}$ to $10^{8.8}$ p.f.u./ml in A. albopictus and A. dorsalis cell cultures, and from $10^{6.5}$ to $10^{7.5}$ p.f.u./ml in C. tarsalis cultures. No decreases in viral titres were observed during the first week after infection (data not shown).

Viral titres in persistently infected cultures were determined at selected times from 2 to 81 weeks p.i. by plaque assay in Vero cells. Although the titres did fluctuate distinct trends were evident in the three pairs of cultures. In Aa1 and Aa2 cultures (Fig. 1a), titres 2 to 3 weeks p.i. ranged from $10^{8.2}$ to $10^{8.8}$ p.f.u./ml. These viral titres gradually decreased to levels more than $10^6$-fold lower than those in the acutely infected cultures: at 81 weeks p.i. the titre of Aa1 virus was $10^{7.5}$ p.f.u./ml and the titre of Aa2 virus was below detectable limits (less than 10 p.f.u./ml). Viral titres decreased from $10^{8.5}$ to $10^{5.5}$ p.f.u./ml in Ad1 cultures and from $10^{7.8}$ to $10^{5.6}$ p.f.u./ml in Ad2 cultures (Fig. 1b). In contrast to the persistently infected Aedes cell cultures, viral titres in Ctl and Ct2 cultures decreased only about 10-fold early in infection and then remained relatively constant at about $10^{6.0}$ to $10^{6.5}$ p.f.u./ml over the remainder of the 80-week period of observation.

The apparent decline in viral titres in Aa1, Aa2, Ad1 and Ad2 cultures might reflect a decreased efficiency of plating on the assay cell line rather than a true decline in numbers of infectious virions. This decreased plating efficiency could possibly be due to the presence of host range (hr) or ts mutants since the plaque assay was performed in another cell type (Vero rather than mosquito) and incubated at a different temperature (37 °C instead of 28 °C). To minimize differences in titre due to possible host or temperature restrictions, selected samples were assayed by IPFA in the homologous mosquito cell lines at 28 °C. As shown in Fig. 2, a significant change in relative plating efficiency was seen in virus from Aa1 and Aa2 cell cultures; titres decreased only $10^{3.9}$- to $10^{4.2}$-fold ($10^{7.7}$ to $10^{4.7}$ p.f.u./ml for Aa1 virus and $10^{7.7}$ to $10^{3.4}$ p.f.u./ml for Aa2 virus) when measured in A. albopictus cells (Fig. 2a) as compared to a $10^{6.6}$- to $10^{7.8}$-fold decrease when measured by plaque assay in Vero cells (Fig. 1a). Plating efficiencies of virus from Ad1, Ad2, Ctl and Ct2 cell cultures were similar in both Vero and the homologous mosquito cells. These results demonstrate that decreases in titre are likely to be due, in part, to decreased production of infectious virus. The results with the A. albopictus cell cultures also suggest that hr or ts mutants may be present in these cultures.

Percentage of cells infected

The percentage of cells containing viral antigens in acutely and persistently infected cell cultures is shown in Table 1. Virtually all cells in acutely infected cultures were virus-positive. At 81 weeks after infection about 70% to 85% of cells in Aa1, Aa2, Ctl and Ct2 cultures produced detectable antigen; however, the proportion of antigen-containing cells in persistently infected Ad1 and Ad2 cultures was somewhat lower, at 41% to 54%. Comparison of average staining densities of infected cells (Table 1) showed that the quantity of viral antigen detected in
Fig. 1. Titres of SLE virus from persistently infected mosquito cell cultures. (a) ●, Aa1; ○, Aa2; (b) ■, Ad1; □, Ad2; (c) △, Ct1; △, Ct2. Viral titres were determined by plaque assay in Vero cells at 36 °C.

Fig. 2. Titres of SLE virus from persistently infected mosquito cell cultures measured in the homologous (mosquito) cell lines. (a) ●, Aa1; ○, Aa2; (b) ■, Ad1; □, Ad2; (c) △, Ct1; △, Ct2. Titres were determined by IPFA at 28 °C as described in Methods.

Table 1. Viral antigens in mosquito cell cultures acutely and persistently infected with SLE virus

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Time p.i. (weeks)</th>
<th>No. cells positive (%)</th>
<th>Relative mean staining density</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. albopictus</td>
<td>1</td>
<td>100</td>
<td>0.24</td>
</tr>
<tr>
<td>Aa1</td>
<td>81</td>
<td>85</td>
<td>0.04</td>
</tr>
<tr>
<td>Aa2</td>
<td>81</td>
<td>70</td>
<td>0.06</td>
</tr>
<tr>
<td>A. dorsalis</td>
<td>1</td>
<td>99</td>
<td>0.21</td>
</tr>
<tr>
<td>Ad1</td>
<td>81</td>
<td>54</td>
<td>0.04</td>
</tr>
<tr>
<td>Ad2</td>
<td>81</td>
<td>41</td>
<td>0.04</td>
</tr>
<tr>
<td>C. tarsalis</td>
<td>1</td>
<td>100</td>
<td>0.14</td>
</tr>
<tr>
<td>Ct1</td>
<td>81</td>
<td>85</td>
<td>0.06</td>
</tr>
<tr>
<td>Ct2</td>
<td>81</td>
<td>79</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Percentage of cells with integrated staining densities greater than 1 S.D. above the mean staining density of uninfected cells.
† The mean density of stain of each cell population was adjusted to relative values: 0, background; 1.0, highest value for an individual cell.
persistent infected cells was substantially less than that found in acutely infected cells and that the degree of decrease was similar in all cell lines. Interestingly, a few (<1%) intensely stained cells were usually observed in all persistently infected cell cultures.

Superinfection with homologous and heterologous viruses

The susceptibility of persistently infected cultures to superinfection with homologous (SLE) virus and heterologous viruses was determined. It should be noted that the superinfection studies with SLE virus were facilitated by the fact that plaques produced by viruses from Aal, Aa2, Ad1, Ad2 and Ct2 cultures were of the sp phenotype and were readily distinguished from those of wt SLE virus; however, virus from Ct1 cultures had a wt plaque phenotype. Aal, Aa2, Ad1 and Ad2 cultures that were superinfected with the LAV5-156 and Parton strains of SLE virus continued to produce only sp virus. Furthermore, the titres of virus produced by these superinfected cultures were similar to those produced by cultures that were not superinfected, and were significantly lower than titres produced by infection of cells that were not persistently infected (Table 2). Thus, these cultures (Aal, Aa2, Ad1 and Ad2) were resistant to superinfection with wt SLE virus. It was not possible to evaluate critically the susceptibility of Ct1 and Ct2 cultures to superinfection with SLE virus because the titres of virus in persistently infected cultures were similar to those produced in acutely infected cells; however, virus produced in superinfected Ct2 cultures was all sp, which suggested that this culture at least was resistant to superinfection.

All persistently infected cultures were susceptible to superinfection by two heterologous flaviviruses, JE and YF. However, the viral titres produced in Aal, Aa2, Ad1 and Ad2 cultures were consistently lower (0.5 to 1.8 log10 p.f.u./ml) than titres produced in cells that were not persistently infected with SLE virus (Table 2), suggesting that some interference with replication in the persistently infected cell cultures may have occurred.

Aal, Aa2, Ad1 and Ad2 cultures were also susceptible to superinfection with WEE virus. Interestingly, titres produced in Aal and Aa2 cultures were slightly higher than those produced in acutely infected A. albopictus cultures (this observation was also noted in two additional experiments (data not shown)). None of the C. tarsalis cell cultures (including the uninfected parental line) could be infected with WEE virus.
TUR virus was able to replicate in all six persistently infected cultures, although the titres in *A. albopictus* cultures were very low. The results were, however, reproducible, and immunoperoxidase staining of infected cells confirmed that replication of TUR virus had indeed occurred (data not shown).

**Curing of persistent infections by virus-specific antibody**

To determine whether persistently infected cultures could be cured by treatment with virus-specific antibody, cells were subcultured in medium containing 5% SLE virus-IMAF. This concentration of SLE virus-IMAF neutralized wt virus as well as virus from persistently infected cultures. Antibody-treated cultures were monitored for infection by immunoperoxidase staining, viral titres and sensitivity to superinfection with homologous virus.

No overt differences between immunostaining of untreated and antibody-treated *Aa1, Aa2, Ad1* and *Ad2* subcultures were seen (Fig. 3a to h) during 12 weeks of antibody treatment; these subcultures were therefore discontinued. In contrast, antibody treatment had a marked effect on *Ctl* and *Ct2* cultures. Untreated *Ctl* and *Ct2* cultures showed a relatively diffuse immunostaining pattern like that seen in acutely infected *C. tarsalis* cultures, whereas antibody-treated cultures were characterized by intensely stained intracytoplasmic vesicles which slowly decreased in number over time (Fig. 3i to l and Fig. 4). The nature of immunostaining in a control *Ctl* culture treated with normal mouse ascitic fluid was unaffected (data not shown). In the *Ctl* culture, cells containing viral antigen were no longer seen after 8 weeks of antibody treatment; however, if grown in antibody-free medium for 1 week these cells once again became antigen-positive (Fig. 4d). The antibody-treated *Ctl* cells were not cured of virus (i.e. they did not remain antigen-negative when antibody was removed from the medium) until they had been treated with antibody for 20 weeks (Fig. 4e). Normal yields of virus were produced when the cured *Ctl* culture was infected with wt SLE virus (Table 3).

The number of immunostaining cells in the antibody-treated *Ct2* culture initially decreased greatly but then began to rise. After 28 weeks of antibody treatment, the staining pattern resembled that of the untreated *Ct2* culture (Fig. 4f to k). Thus, even after 28 weeks of treatment with antibody it appeared that the *Ct2* culture could not be cured. It is interesting to note that after approximately 19 weeks of antibody treatment, transient c.p.e. was seen in three successive weekly subcultures which were passaged in the absence of antibody. Cytopathology appeared approximately 6 to 7 days after cells were passaged, and consisted of large areas of cellular degeneration and syncytium formation. Regeneration of the monolayer occurred rapidly each time and was complete within 1 week.

Although the *Ct2* culture remained antigen-positive following antibody treatment (Fig. 4), virus could not be detected by plaque assay or IPFA, possibly because extracellular virus was neutralized by the antibody still present in the culture medium. Following one passage in antibody-free medium, however, viral titres as assayed by IPFA in normal *C. tarsalis* cells rose to levels similar to those in the untreated *Ct2* cultures (Table 4). In contrast, when virus from the antibody-treated *Ct2* culture was assayed in Vero cells, the titre was 1000-fold lower than in *C. tarsalis* cells. The lower efficiency of plating of this virus population on Vero cells compared to *C. tarsalis* cells suggests that antibody treatment may have selected for *hr* mutants.

**DISCUSSION**

Previous studies have demonstrated that productive, non-cytocidal infections are readily established in arbovirus-infected mosquito cell cultures. In the present study we have characterized and compared persistent infections with SLE virus in three different mosquito cell lines (*A. albopictus, A. dorsalis* and *C. tarsalis*). The three persistently infected cell lines were found to be similar, in that no overt c.p.e. was observed and the percentage of infected cells remained relatively high, although by IPFA a larger fraction of *A. albopictus* and *C. tarsalis* cells appeared to be infected (>70%) as compared to *A. dorsalis* cells (>40%). Similar to previous reports, the *Aedes* cultures became resistant to superinfection by homologous but not heterologous viruses. (Superinfection of the *C. tarsalis* cultures could not be evaluated.)
Fig. 3. Photomicrographs of antibody-treated and untreated mosquito cell cultures persistently infected with SLE virus. Cultures were persistently infected with SLE virus for 99 weeks and subcultures were treated with SLE virus-IMAF for 8 weeks. Cells were stained by an immunoperoxidase method using SLE virus-immune rabbit serum as the primary antibody. (a) Aa1, (b) Aa1 + SLE virus-IMAF, (c) Aa2, (d) Aa2 + SLE virus-IMAF, (e) Ad1, (f) Ad1 + SLE virus-IMAF, (g) Ad2, (h) Ad2 + SLE virus-IMAF, (i) Ct1, (j) Ct1 + SLE virus-IMAF, (k) Ct2, (l) Ct2 + SLE virus-IMAF.

Although viral titres in persistently infected *A. albopictus* and *A. dorsalis* cell cultures decreased considerably over time, relatively large amounts of viral antigens, as detected by immunostaining, continued to be produced. This suggests that a significant amount of defective
or non-infectious virus was being produced. Additionally, titres of infectious virus in *A. albopictus* cultures were significantly higher when assayed in the homologous mosquito cell line (*A. albopictus*) at 28 °C than in Vero cells at 37 °C, suggesting that the infectious virus might be either temperature-sensitive or host-restricted. In contrast to the situation in persistently
SLE virus persistent infections

Table 3. Superinfection of cured Ctl cultures with SLE virus*

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Antibody-treated</th>
<th>Mock-infected</th>
<th>Superinfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. tarsalis</td>
<td>-</td>
<td>&lt;1·0</td>
<td>6·5</td>
</tr>
<tr>
<td>Ctl</td>
<td>-</td>
<td>5·7</td>
<td>6·3</td>
</tr>
<tr>
<td>Ctl</td>
<td>+</td>
<td>&lt;1·0</td>
<td>7·2</td>
</tr>
</tbody>
</table>

* Ctl cultures were 119 weeks p.i. Cultures had been treated with SLE virus-IMAF for 29 weeks and then subcultured for 3 weeks in antibody-free medium before superinfection.
† Titres were determined by plaque assay in Vero cell cultures.

Table 4. Comparison of SLE viral titres produced by antibody-treated and untreated Ct2 cultures*

<table>
<thead>
<tr>
<th>Antibody treatment†</th>
<th>Viral titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vero cells</td>
</tr>
<tr>
<td></td>
<td>(log_{10} p.f.u./ml)</td>
</tr>
<tr>
<td>-</td>
<td>5·4</td>
</tr>
<tr>
<td>+</td>
<td>2·4</td>
</tr>
</tbody>
</table>

* Ct2 cultures were 110 weeks p.i.
† Cultures had been treated with antibody for 24 weeks, then subcultured in antibody-free medium for 1 week.

infected Aedes cells, the small reduction in titres in the C. tarsalis cultures was correlated with a slight reduction in the amount of viral antigen detected, suggesting that these cultures continued to produce infectious virus that was similar to wt virus. These results indicate that long term SLE virus infection in Aedes cells, particularly A. albopictus cells, appeared to favour production of defective virus, whereas long-term infection of C. tarsalis cells favoured production of relatively stable infectious virus.

The effect of antibody on flavivirus-infected mosquito cells has not previously been described. Antibody had no detectable effect on the persistently infected Aedes cell lines, but caused a significant decrease in virus production in the C. tarsalis cell cultures. Only one culture (Ctl) was actually cured; in the Ct2 culture virus production initially decreased greatly, but after several months the culture did not respond to antibody treatment. Two groups have reported that alphavirus-infected A. albopictus cells can be cured by virus-specific antibody (Igarashi et al., 1977; Riedel & Brown, 1977). This was thought to occur by preventing re-infection of 'self-cured' cells, which implies that constant re-infection of cells with virus is necessary to maintain long-term infections. The mechanism by which individual cells are actually cured is unknown and may be related to the regulation of viral replication as has been suggested by Igarashi et al. (1977). We speculate that individual C. tarsalis cells may be capable of 'self-cure' whereas Aedes cells are not. Therefore, persistent infection in C. tarsalis cultures may be maintained by re-infection of 'self-cured' cells, whereas persistent infection in Aedes cells may be maintained by intracellular passage of virus to daughter cells during cell division without a significant role for re-infection of cells by extracellular virus. Thus, regulation of SLE viral replication in the two cell types may be substantially different.

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