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CHRONIC ALPHAVIRUS INFECTION OF L CELLS

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Summary. — Chronic infection of L cells with Sindbis virus, induced in the absence of antiviral antibody, was studied by virological methods in combination with light and electron microscopy. Both Sindbis virus and endogenous oncovirus antigens were revealed in the cells by immunofluorescence. Sindbis virus and interferon were detected in the culture fluid. Immunoelectron microscopy revealed aggregation of Sindbis virus particles by immune serum.

Key words: Sindbis virus; Alphavirus; endogenous oncovirus: L cells; interferon

Introduction

In recent years, great attention has been paid to investigations on chronic infections of cell cultures by a variety of viruses. But numerous problems concerning the mechanisms supporting long-term persistence of viral and cell populations still remain obscure. Inadequate and contradictory have been the reports on the role of antigenic changes of infected cells and of the immunity factors in the development of chronic viral infections (Andzhaparidze and Bogomolova, 1974; Gavrilov *et al.*, 1974; etc.).

The aim of the present work was to study the peculiarities of infection and the cell changes caused by Sindbis virus in mouse L cells.

Materials and Methods

Cell cultures. Monolayer cultures of continuous mouse L cells and strain No. 431 of Chinese hamster (CH) cells were used Primary trypsinized mouse and chick embryo cell cultures were employed for virus and interferon titrations.

Viruses. Sindbis virus strain EgAn No. 339 was used in the form of mouse brain suspension with a titre of $10^{7,5}$ TCD_{50}/ml; vesicular stomatitis virus strain Indiana in the form of infected allantoic fluid had a titre from 10^5—10^7 TCD_{50}/ml.

Table 1. Titration and detection by immune electron microscopy of Sindbis virus in CH cell cultures

Inoculum	Virus titre log TCD_{50}/ml	Immune electron microscopy
Medium from L-cell cultures	—	—
Medium from the 35th passage of L_s-cell cultures	5.7	+
Original Sindbis virus	5.0	+
Mixture of original Sindbis virus with medium from the 35th passage of L_s cells	5.0	+

— means negative result.

Sera. Immune serum to Sindbis virus obtained by immunization of rabbits had titres from 640 to 1280 against 8 antigen units in the haemagglutination inhibition test. Goat immune serum to Gross leukaemia virus was kindly supplied by Dr. X. Wilsnack, Huntingdon Research Center, USA. Moreover we used fluorescein isothiocyanate-labelled rabbit serum to sheep globulins and donkey serum to rabbit globulins produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, U.S.S.R. Academy of Medical Sciences, Moscow.

Electron microscopy. Cell cultures were washed with 0.1 M cacodylate buffer pH 7.2, fixed in 2.5% glutaraldehyde in the same buffer and post-fixed with a 2% OsO_4 solution. The preparations were dehydrated through an ethanol series and embedded into epoxy resin. The virus was visualized by immune electron microscopy (Almeida and Waterson, 1969; Doane, 1974) based on aggregation of virions by homologous immune serum. The preparations were examined in a JEM-100 B electron microscope.

The methods of cell cultivation, immune cytolysis, interferon assay and visualization of viral antigens were those described (Karmysheva *et al.*, 1974; Ovsyannikova and Karmysheva, 1980). The lysed cell counts were subjected to statistical evaluation using nonparametric methods (Zaks, 1976)

Results

The L-cell culture inoculated once with a dose of 5—15 TCD_{50} of Sindbis virus per cell, designated L_s culture, was examined in the course of 65 passages. In the course of the whole experiment, infectious virus was regularly demonstrated in the culture fluids in titres from $10^{2.3}$ to $10^{4.5}$ TCD_{50}/ml, which suggested the persistence of chronic infection. The morphology of the L_s culture and its susceptibility to vesicular stomatitis virus remained unchanged. We found no significant differences in the proliferative and mitotic activities of L- and L_s-cell cultures.

Immunofluorescence revealed Sindbis virus antigen in both native and fixed preparations of L_s cultures. In native preparations, viral antigen was localized in the cell plasmalemma in the form of thin rings, granules or caps (Figs 1 and 2). In fixed preparations, considerable accumulations of diffuse or granular viral antigen were observed in the cytoplasm of 60—70% of the cells (Figs 3 and 4).

Table 2. Median values of the numbers of lysed cells in L_S-cell cultures as revealed by virus-specific immune cytolysis

L_S-cell passage No.	Median % values of cells lysed with immune sera to viruses I Sindbis	II Gross	III Sindbis + Gross (1 : 1)	Wilcoxon criterion
19	62	60	86	
23	6.6	4	8.6	
25	Not done	17.8	76	$P_{I \text{ and } III} = 0.99$
31	25	48	52	$P_{II \text{ and } III} = 0.99$
33	22	62	72	

We examined ultrathin sections of L_S cultures at the 2nd, 6th, 8th, 12th and 18th passage levels. In spite of the numerous preparations examined we failed to detect Sindbis virus in the sections although, as mentioned above, it was regularly present in the culture fluids.

Immune electron microscopy of the culture fluid revealed groups each of 2—3 particles aggregated by immune serum to Sindbis virus. By their morphology and size (25—26 nm) these particles corresponded to nucleocapsids. Occasionally there occurred single particles surrounded by antibody and measuring 50 nm in diameter.

Inoculation of CH cell cultures with culture fluid from the 35th passage of L_S cells resulted in the development of marked cytopathic changes. After 24 hr, when a cytopathic effect had involved more than 50% of CH cells, we examined the material by electron microscopy to detect cell-associated Sindbis virus. To this end, homologous immune serum was added to disrupted CH cells. In such preparations, electron microscopy revealed mature virions aggregated by immune serum (Fig. 5).

To demonstrate the eventual formation of interfering viral particles, CH cell cultures were inoculated with original Sindbis virus, virus from the 35th L_S-cell culture passage or their mixture. All these inocula produced a marked cytopathic effect at the same intervals (Table 1). We thus failed to demonstrate interfering particles in the population of Sindbis virus responsible for the chronic infection of the L_S culture.

All ultrathin sections of L and L_S cell cultures revealed large amounts of endogenous oncovirus particles, mainly mature and immature type C particles, localized extracellularly as well as budding from the plasma membranes. The type C particles were especially numerous in L_S-cell cultures (Figs 6 and 7). By immunofluorescence with highly specific hyperimmune serum to Gross virus we found in both native and fixed preparations that the antigen of endogenous virus was present in all cells of both the original L culture and the L_S culture (Figs 8—11). But L_S cells showed a more in-

tensive fluorescence suggesting greater accumulation of antigens of the endogenous virus.

Results concerning the detection of virus-induced antigens in the plasmalemma by the method of immune cytolysis (Table 2) showed that virus-specific immune cytolysis occurred in L_s cultures regularly after addition of both immune serum against Sindbis virus and immune serum to the oncovirus, although the number of lysed cells varied from one passage to another. The proportion of lysed cells adsorbing homologous antibody in amounts sufficient for the activation of complement was considerably lower than the proportion of antigen-containing cells revealed by immunofluorescence. Oncovirus-induced surface antigens demonstrable by the cytolytic test were regularly observed also in the uninfected control L-cell culture, but the number of lysed cells in the latter was not higher than 18% as compared with 62% in some passages of L_s-cell cultures. The number of lysed cells increased when a mixture of both antisera to Sindbis and Gross viruses was used.

Interferon was not found in culture fluids from L-cell cultures. The examined samples of fluids from L_s-cell cultures contained interferon in titres up to 40 units per ml.

Discussion

The opinions about the formation and maintenance of persistent infections vary depending on a number of factors like cell type, presence or absence of interferon, antibody, virus mutants, defective interfering particles, etc. Several authors used antiviral antibody in inducing chronic infection of various L-cell strains with Sindbis virus. For example Azadova *et al.* (1972) passed a Sindbis virus-infected L-cell culture in the presence of antiviral antibody. Infectious virus was detected in low titres only in the course of the first 2—3 weeks after inoculation. Interferon was not found in the culture fluid. These authors assumed that persistent infection led to the formation of defective virions which can be transmitted only from cell to cell.

In our system L cells — Sindbis virus a chronic infection developed without addition of specific antiviral antibody. We failed to detect defective interfering particles. The morphology of L_s-cell culture, its growth and mitotic activity did not differ from those of L-cell cultures. Considerable amounts of viral antigen accumulated in the cytoplasm and titres of extracellular virus reached $10^{4.5}$ TCD_{50}/ml. No Sindbis virions were observed in ultrathin sections. Occasional virus particles were demonstrated by immune electron microscopy in culture fluid from L_s cells. Transfer of medium from the 35th passage of L_s cells into CH cell cultures led to the development of an acute infection.

Zhdanov *et al.* (1978) also failed to demonstrate Sindbis virus by electron microscopy in ultrathin sections of a chronically infected L-cell culture. These authors, however, regularly detected the virus in sections of brains

from mice inoculated intracerebrally with a cell homogenate or preparations of DNA extracted from these cultures. These data indicate that the amounts of Sindbis virus in chronically infected L-cell cultures are very low.

Andzhaparidze and Bogomolova (1973) are of the opinion that the basic factor responsible for maintenance of a carrier state is the presence of interferon or development of autointerference. A number of authors confirmed the importance of interferon in maintenance of chronic infection. Stanček (1965) found that lowering of the temperature of incubation of L cells to 30 °C led to a virus titre increase and death of the culture due to lowered interferon production by the cells. Inglot *et al.* (1973) showed that highly specific anti-interferon globulin which neutralizes interferon caused, upon addition into the medium of L-cell cultures, a considerable increase in Sindbis virus synthesis leading to destruction of the culture. We found interferon in all samples of fluids from L_s-cell cultures examined. This fact evidently was one of the factors responsible for the establishment and maintenance of a chronic infection in our system. The presence of an endogenous oncovirus also could have played a role.

It appears that in our system the establishment of chronic Sindbis virus infection was supported by a number of factors: lowered susceptibility of mouse cells to the causative agent; formation of interferon; stimulation of endogenous oncovirus; and accumulation of considerable amounts of viral antigens along with negligible formation of infectious virus.

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Explanation of Micrographs (Plates X—XII):

Figs 1—4. Surface (*1, 2*) and intracellular (*3, 4*) antigens of Sindbis virus in L_s cells. Indirec immunofluorescence.

Fig. 5. Sindbis virus particles aggregated by homologous immune serum. A single virion covere by antibody is shown in the bottom left corner. × 150 000.

Figs 6 an *7.* Particles of endogenous oncovirus — mature (*6*) and budding form the cytoplasmi membrane (*7*). × 66 000.

Figs 8—11. Surface (*8, 9*) and intracellular (*10, 11*) antigens of endogenous oncovirus in L cells (*8, 9, 11*) and L cells (*10*). Indirect immunofluorescence.