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POTISKUM VIRUS: ENHANCEMENT OF REPLICATION IN A MACROPHAGE-LIKE CELL LINE

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Summary. — Replication of Potiskum virus was studied in P388D_1 macrophage-like cell line in the presence and absence of subneutralizing concentrations of specific antiviral antibody. The cultures were infected at multiplicities of infection (MOI) ranging from 0.4 to 0.0004. The virus replicated to high titres at all MOI tested, but there was an enhancement of virus replication in cultures supplemented with the antibody. Enhancement of replication was MOI dependent, the highest ratios being obtained in cultures infected at lowest MOI. In enhancement assays using various dilutions of immune mouse ascitic fluid (IMAF), the highest enhancement ratio was observed at dilution 1 : 500; the enhancing antibody titre was 5,000.

Key words: Potiskum virus; neutralizing antibody; enhancement; macrophage-like cells

Introduction

The replication of several flaviviruses in macrophage-like cell lines can be enhanced in the presence of subneutralizing concentrations of antibody (Peiris and Porterfield, 1979; Schlesinger and Brandriss, 1981; Halstead *et al.*, 1983). The phenomenon of immunological enhancement of virus infections, which is a result of interaction between virus, Fc receptor bearing cells and antibody has been incriminated as a possible pathogenetic mechanism in the severity and acceleration of certain virus diseases (Halstead *et al.*, 1982; Porterfield, 1982).

Over seventy flaviviruses have been isolated from humans, wild and domestic animals and mosquitoes. Immune enhancement phenomenon has been demonstrated with only a few flaviviruses, namely the West Nile, dengue type 2, Uganda S, louping ill, Japanese encephalitis, yellow fever and Murray Valley encephalitis viruses (Peiris and Porterfield, 1979; Peiris and Porterfield, 1981; Schlesinger and Brandriss, 1981; Halstead *et al.* 1983; Kliks and Halstead, 1983). In this paper, the results of immunological enhancement of Potiskum virus infection in P388D_1 cells are presented. Potiskum, a hitherto unknown flavivirus, was isolated in 1968 in Nigeria

from *Cricetomys gambianus* (African pouched rat) during a routine virus surveillance. Serological studies showed that it is closely related to Uganda S and Banzi viruses (Theiler and Downs, 1973).

Materials and Methods

Virus. A ninth mouse brain passage of Potiskum virus was used in enhancement assays. Virus seed was prepared as 10% suckling mouse brain (SMB) suspension in phosphate buffered saline (PBS pH 7.95) containing 0.5% gelatin. Virus titre was 2×10^7 plaque forming units (PFU) per ml in baby hamster kidney (BHK-21) cells.

Virus titration. Assay for virus infectivity was carried out by plaque assay in BHK-21 cells. Virus suspension (0.05 ml) was inoculated in triplicate into 24 well tissue culture plastic plates. Virus adsorption was allowed to proceed for 60 min at 37 °C, with occasional shaking at 15 min intervals. Monolayers were subsequently overlaid with carboxyl methyl cellulose (CMC) overlay containing 3% CMC-sodium salt (medium viscosity), BME Hanks, foetal calf serum and sodium bicarbonate. Cultures were incubated at 37 °C in 5% carbon dioxide and stained with naphthol blue black in acetic acid 3 days later.

Immune mouse ascitic fluid. Immune mouse ascitic fluid (IMAF) was prepared by single injection of adult Swiss Webster mice with a mixture of virus and Freund's complete adjuvant as described by Brandt *et al.* (1967).

Antibody determinations. Potiskum virus IMAF was tested for neutralizing (N) antibody by a micro plaque reduction neutralization test (PRNT) in BHK-21 monolayers. One tenth ml of virus suspension calculated to give a dose of 40 PFU/0.05 ml was added to 0.1 ml of various dilutions of IMAF. Virus-ascitic fluid mixtures were incubated at 37 °C for 1 hr and then inoculated into BHK monolayers in 24 well tissue culture plates. Monolayers were incubated at 37 °C in 5% CO_2 for 1 hr shaking every 15 min and overlaid with CMC overlay. Cultures were stained with naphthol blue-black 3 days later. Plaques were counted and the PRNT antibody titres was determined as the dilution of IMAF reducing the number of plaque in the control well by 50%. The antibody titre was 1 : 20.

Cultivation of cells. A methyl cholanthrene induced mouse tumor cell line $P388D_1$ was used. These cells possess several macrophage characteristics including macrophage-like morphology, immune phagocytosis the Fc and C3 receptors (Peiris and Porterfield, 1979). The cells were grown in MEM-alpha (Gibco) with 10% foetal calf serum (FCS), 200 μg of streptomycine and 200 units of penicillin per ml and incubated at 37 °C in 5% CO_2. Cells were cultivated by dispersing monolayers with freshly made growth medium using a 20 ml syringe and a 14 gauge cannula. Cultures were seeded at a concentration of 3×10^5 per ml in 75 cm^2 plastic tissue culture flask and formed monolayers in 3—4 days.

Growth curve studies. The kinetics of growth of Postiskum virus was investigated in $P388D_1$ cells at 4 different multiplicities of infection (MOI): 0.4, 0.04, 0.004 and 0.0004. Cultures were seeded at 5×10^5 cells per 0.9 ml in MEM-alpha and virus dilution which produced the required MOI was added. Nine tenths ml per well infected cells were dispensed into 24 well plastic tissue culture plates containing 0.1 ml of 1 : 500 of IMAF or normal immune ascitic fluid (NMAF). Infected cultures were incubated at 37 °C in 5% CO_2 and harvested daily for 4 days. Whole cultures were frozen at −70 °C; the assay of thawed cultures was carried out by plaquing on BHK-21 monolayers under CMC overly. Enhancement ratio was determined by dividing the mean number of plaques in antibody-supplemented cultures by that formed in cultures containing NMAF.

Enhancing antibody assay. Potiskum IMAF was titrated for enhancing antibody at 1 : 50, 1 : 500, 1 : 5,000 and 1 : 50,000. The day and the MOI producing the highest enhancement ratios in the growth curve experiments was selected for enhancing antibody assays. Cultures were seeded as above and infected at the selected MOI and supplemented with various dilutions of IMAF or NMAF. Assay of infected cultures was carried out and the enhancement ratio produced by each dilution was determined. The enhancing antibody titre was the highest dilution producing significant enhancement of infection in culture when tested by the formula of Detre and White (1970):

$$X_1 - X_0 / \sqrt{X_1 - X_0} \geq 1.96$$

where X_1 — total of plaque counts in antibody supplemented cultures and X_0 — total plaque conts in same number of control cultures.

Results

The growth curves of Potiskum virus in P388D$_1$ cells in the presence and absence of antibody are shown in Fig. 1. Virus replication occurred in both antibody supplemented and non-supplemented cells at all MOI tested. Higher virus titres were found in the cultures containing antibody. When infected with higher MOI (0.4) virus replication was first detected on day 1; virus titres varied between 2×10^4 PFU/ml and 2×10^6 PFU/ml in antibody--supplemented cultures and 2×10^3 and 1.2×10^5 PFU/ml in non-supplemented cultures. Cultures infected at the lowest MOI had a late virus growth. Virus replication was detected on day 2 p.i. and on day 3 p.i. in antibody supplemented and antibody non-supplemented cultures, respectively. Peak virus titres were 1×10^6 PFU/ml in cultures containing antibody and 4×10^3 in the controls.

Enhancement ratio was MOI dependent, highest enhancement ratios were obtained in cultures infected at the lowest MOI. The peak enhancement

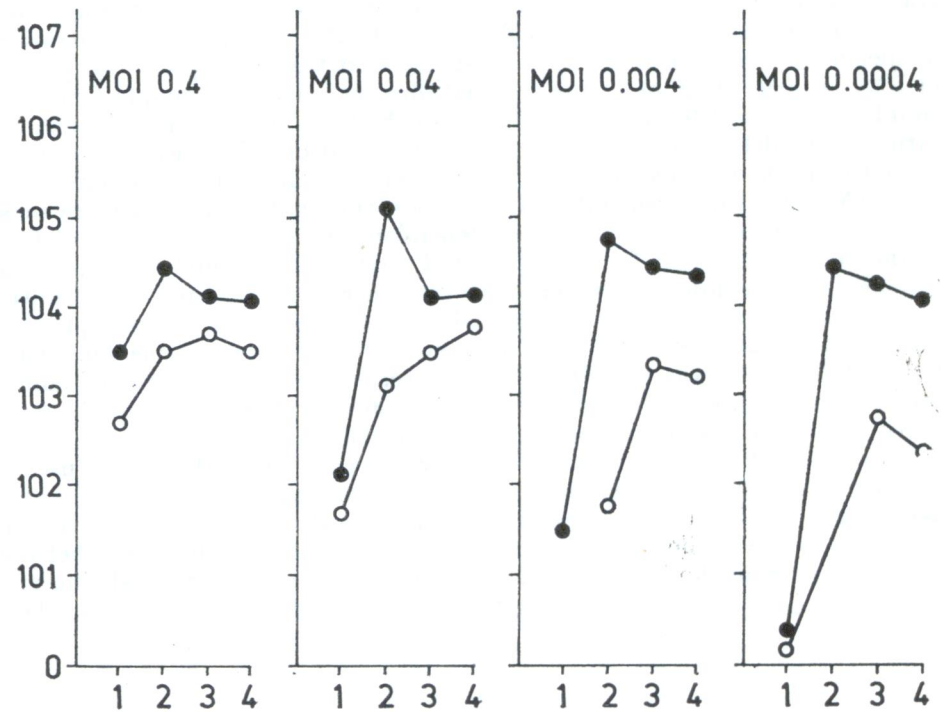

Fig. 1.
Growth curves of Potiskum virus in P388D$_1$ cultures. Tested in the presence (●——●) or absence of (○——○) subneutralizing concentrations of homologous antibody. Antibody and NMAF were added at 1 : 500 dilutions at all MOI's tested.
Abscissa: virus titres (PFU/0.5 ml); ordinate: days in culture.

ratio obtained on day 2 p.i. and ranged from X1,000 (in cultures infected at MOI of 0.0004) to X8 (in cultures infected at 0.4 MOI).

Severe cytopathic effect (CPE) was found in antibody supplemented cultures on day 3 and 4 p.i.; low grade CPE was detected in control cultures on day 4 p.i.

Potiskum virus IMAF produced enhanced virus replication in culture at dilutions ranging between 1/50 and 1/5,000. Highest enhancement ratio was obtained at 1 : 500 dilutions. Dilutions below 1/50 and above 1/5,000 failed to produce any enhancement of infection.

Discussion

The present study showed that Potiskum virus, a new flavivirus (Theiler and Downs, 1973) which has not been previously propagated in cell culture, replicated to high titres in a macrophage-like cell line. In addition to the other flaviviruses studied (Peiris and Porterfield, 1979; Halstead *et al.*, 1983; Halstead and Kliks, 1983; Schlesinger and Brandriss, 1981), Potiskum virus infection can also be enhanced in $P388D_1$ cell cultures by low concentrations of specific antiviral antibody. It was shown that this virus produced plaques in $P388D_1$ cells (Fagbami, unpublished observations) and plaque formation could be enhanced by non neutralizing antibody, however, the enhancement ratio obtained by enhancement of plaque formation (X44) was much lower than the peak enhancement ratio obtained by enhancement of infectivity titres (X1000). Enhancement of virus replication is, therefore, a more sensitive method of measuring antibody dependent enhancement (ADE) of Potiskum virus infections. It was suggested that immune enhancement is an opsonization phenomenon which facilitates entry of virus into cells resulting in increase in the number of infected cells and hence of virus replication (Halstead, 1982). The immune enhancement of Potiskum virus as demonstrated in this study was manifested by increase in virus content of culture fluids supplemented with subneutralizing concentrations of antibody or by early onset of virus multiplication in such cultures.

Porterfield *et al.* (1981) had previously shown that ADE can be used as a tool for measuring antibody and found it as sensitive as radioimmunoassay and 10 times more sensitive than neutralization test in measuring antibody to West Nile and Uganda S viruses. The marked difference between the enhancing antibody titres (1 : 5,000) and conventional neutralizing antibody titre (1 : 20) further reveals the sensitivity of the former in antibody assay.

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