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Antibody-mediated enhancement of Wesselsbron virus in P388D$_1$ cells

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Summary

Antibody-mediated enhancement of Wesselsbron virus was investigated in P388D$_1$ cell cultures. Virus infection was enhanced in culture by various dilutions of homologous and heterologous flavivirus antibody. Highest enhancement ratios and enhancing antibody titres were obtained with the homologous antibody.

Enhancement of Wesselsbron virus infection in P388D$_1$ cultures was also dependent on the multiplicity of infection (MOI) used; cultures infected at the lowest MOI produced the highest enhancement ratios. Of the four heterologous flavivirus IMAF tested for ability to enhance Wesselsbron virus infection, Potiskum virus antibody produced highest fold enhancement and possessed the highest enhancing antibody titre. Zika, Uganda S and Dakar bat IMAF produced lower fold enhancement and had lower enhancing antibody titres.

Résumé

L'accroissement par l'intermédiaire des anticorps, du virus Wesselsbron, fut étudié au moyen des cultures effectués dans les cellules P388D$_1$. La quantité du virus infectueux fut accrue dans la culture en dilutions variées de l'anticorps flavivirus homologue et hétérologue. Le taux d'accroissement le plus haut et la quantité d'anticorps capable de multiplier le virus furent obtenus avec de l'anticorps homologue. L'accroissement du virus infectueux dans les cultures P388D$_1$ dépendait également de la multiplicité de l'infection (MOI) utilisée; les cultures infectées au niveau (MOI) le plus bas avait produit le taux d'accroissement le plus haut. Parmi les quatre IMAF flavivirus hétérologues testés pour leur capacité d'accroitre le virus infectueux Wesselsbron, l'anticorps virus Potiskum avait produit la quantité d'accroissement la plus haute, et il avait la plus grande proportion d'anticorps susceptible de provoquer l'accroissement. Zika, Uganda S et Dakar avaient produit d'accroissement la plus basse et ils avaient la plus petite proportion d'anticorps susceptible de provoquer l'accroissement.

Introduction

Enhanced replication of several flaviviruses has been demonstrated in macrophages derived from human and simian peripheral blood (Halstead & O'Rourke, 1977; Halstead *et al*., 1976) Recently, West Nile, dengue and yellow fever viruses were shown to replicate in macrophage-like cell lines and exhibit immune enhancement (Peiris & Porterfield, 1979; Schlesinger & Brandriss, 1981; Halstead *et al*., 1983). Immune enhancement is a biological phenomenon that requires interaction between virus and Fc receptor bearing cells in the presence of subneutralizing concentrations antibody. This phenomenon has been postulated to account for increased severity or early death in certain diseases (Halstead, 1982; Porterfield, 1981) and has been established as a major mechanism in the pathogenesis of dengue haemorrhagic fever/shock syndrome (DHF/DSS).

There are many other important viruses of man and animals that have not been studied with regards to immune enhancement phenomenon. In this paper, therefore, the results of the study of immunological enhancement of Wesselsbron virus (a flavivirus responsible for human and animal disease in Africa) in P338D$_1$ cell line are presented.

This cell line is derived from a methyl

cholanthrene-induced mouse tumour and exhibits macrophage-like characteristics including macrophage-like morphology, immune phagocytosis, FC and C3 receptors and production of lysozyme.

Viruses

Wesselsbron virus stock used in enhancement assays, was made in C6/36 cell culture. The virus was the prototype strain which had previously undergone two serial intracerebral (i.c.) passages in suckling mice and subsequently passaged twice in C6/36 cells. Virus stock was aliquoted in 0.5 ml and stored at −70°C. The infectivity titre was 2×10^6 plaque forming units (pfu) per ml in LLC-MK2. Details of viruses used in preparing immune ascitic fluids are shown in Table 1.

Immune mouse ascitic fluids

Immune mouse ascitic fluids (IMAF) were prepared against Wesselsbron and four related flaviviruses namely: West Nile, yellow fever, Potiskum and Zika viruses in Swiss Webster mice. Adult mice were inoculated with one injection of a mixture of 10% suckling mouse brain (SMB) suspension and Freunds complete adjuvant as decribed by Brandt, Buescher and Metrick (1967). Mice were subsequently inoculated with 0.5 ml of sarcoma T.G. 180 intraperitoneally 7–10 days post-immunization and tapped by paracentesis 10–12 days later.

Neutralizing antibody determinations

Immune mouse ascitic fluids were heat inactivated at 56°C for 30 min and tested for neutralizing (N) antibody by the standard plaque reduction neutralization test (PRNT) in LLC-MK2 monolayers. Neutralizing antibody titres were expressed as reciprocals of dilutions of IMAF producing a 50% reduction in number of plaques (Table 2).

Cultivation of P388D$_1$ cells

The procedure for cultivation of P388D$_1$ cells has been described by Halstead *et al.* (1983).

Table 1. Strains and passage history of viruses used for preparing immune mouse ascitic fluids

Virus	Strain	Passage level	Host system
Zika	East African	5	LLC-MK-2/Suckling mice
West Nile	East African	3	LCC-MK-2/Suckling mice
Potiskum	Ib-An 10069	9	Suckling mice
Yellow fever	17D	1	Suckling mice
Wesselsbron	South African	2	Suckling mice

Table 2. Plaque reduction-neutralization titres of 1-injection flavivirus immune ascitic fluids

	Viruses				
IMAF	Wesselsbron	Potiskum	West Nile	Yellow fever	Zika
Wesselsbron	*40*	<10	<10	<10	<10
Potiskum	<10	*20*	<10	<10	<10
West Nile	<8	<8	*8*	<8	<8
Yellow fever	<10	<10	<10	*10*	<10
Zika	<10	<10	<10	<10	*40*

Briefly, cells were grown in MEM-alpha medium (Gibco) with 10% foetal calf serum (FCS), 200 μg streptomycin and 200 units penicillin per ml, and incubated at 37°C in 5% carbon dioxide. Cells were dispersed with fresh growth medium using a 20-ml syringe and a 14 gauge cannula and seeded at a concentration of 3×10^5/ml in 75 cm^2 plastic tissue culture flasks.

Enhancement assays in P388D$_1$

For enhancement assays, P388D$_1$ cells were suspended at 5×10^5/ml in complete MEM-alpha medium, and virus dilution, which produces the required multiplicity of infection (MOI), was added. Infected cells were dispensed at 0.9 ml per well into 24-well plastic tissue culture plates containing either 0.1 ml of 1:500 dilution of heat inactivated IMAF or normal mouse ascitic fluid (NMAF) per well. Plates were incubated at 37°C in 5% CO_2 and harvested for 4 days. Whole cultures were frozen down at −70°C and thawed for assay. Cultures were assayed in LLC-MK2 in triplicate.

Fold enhancement or enhancement ratio was determined by dividing plaque counts in IMAF supplemented cultures by plaque counts in control cultures containing NMAF. Enhancing antibody titre was defined as the highest dilution of IMAF that produced at least ×2 fold-enhancement of virus infection in culture.

Virus assay

Assay of infected cultures was performed by plaquing in LLC-MK2 cells in 24-well tissue culture plastic plates. Ten-fold serial dilutions of freeze-thawed cultures were made in PBS-gelatin pH 7.95 and 0.05 ml was inoculated in triplicate wells. Virus adsorption was allowed to proceed at 37°C for 60 min with occasional shaking at 15-min intervals. Cultures were then overlaid with 1% noble agar containing BME-Hanks without phenol red, 10% calf serum, 200 μg streptomycin and 200 units penicillin per ml, 20 M Hepes buffer and neutral red. Plates were incubated at 37°C in 5% CO_2. Plaques were counted at 7 days and 14 days.

Results

Enhancement assays on Wesselsbron virus were carried out at 4 different MOI: 0.1, 0.01, 0.001 and 0.0001 in P388D$_1$ cultures supplemented with 1:500 dilution of Wesselsbron IMAF or NMAF. Virus growth occurred in both antibody supplemented and non-antibody supplemented cultures at all MOIs tested (Fig. 1). Higher virus titres were consistently detected in cultures containing antibody than in those without. At the highest MOI (0.1) high virus titres were detected on day 1 post-infection (p.i.) in the antibody supplemented cultures and the controls. Peak fold enhancement (×7) also occurred on day 1 p.i. In contrast, cultures infected at the lowest MOI (0.0001) had late virus growth and higher peak fold enhancement. Virus was detected in test cultures on day 2 p.i. and on day 3 p.i. in the controls; peak fold enhancement of ×56 was obtained on day 3 p.i.

Enhancement of Wesselsbron virus was also carried out at four dilutions (1:50, 1:500,

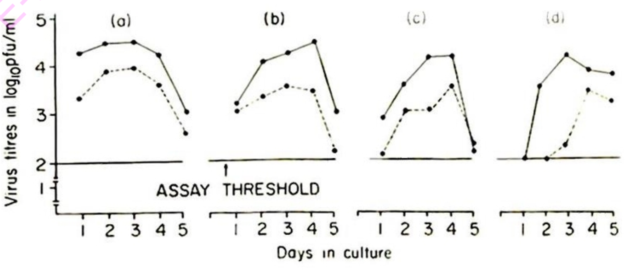

Fig. 1. Growth curves of Wesselsbron in P388D$_1$ cultures with (●———●) or without (●- - - - -●) homologous antibody. Virus was added at different MOIs (a) 0.1 (b) 0.01 (c) 0.001 (d) 0.0001. Antibody and NMAF were added at 1:500 dilutions.

1:5,000, 1:50,000) of homologous and four heterologous IMAF using the virus at the MOI (0.0001) producing the highest enhancement ratio. Peak fold enhancement was produced by 1:500 dilution of homologous IMAF (Table 3). Heterologous flavivirus IMAF to Zika, yellow fever, West Nile and Potiskum viruses (a new flavivirus isolated from *Cricetomys gambianus*, African pouched rat (Theiler & Downs, 1973) also enhanced Wesselsbron virus infection in culture. Lower fold enhancement and enhancing antibody titres were obtained with all heterologous IMAF. Of the four heterologous flavivirus IMAF tested for enhancement capacities, Potiskum IMAF produced the highest fold enhancement ×8.5 and enhancing antibody titre (Table 3). Peak fold enhancement and enhancing antibody titres obtained with yellow fever, Zika and West Nile antibodies ranged from ×6 to ×3 and 1:50 to 1:500, respectively.

The present enhancement assays in P388D$_1$ cells showed that Wesselsbron, like other flaviviruses previously studied (Peiris & Porterfield, 1979; Schlesinger & Brandriss, 1981; Halstead *et al.*, 1983), can be enhanced in culture by sub-neutralizing concentrations of homologous and heterologous flavivirus antibody. The higher infection enhancement capacity of homologous immune mouse ascitic fluid observed in this and previous studies (Peiris & Porterfield, 1979) can be attributed to the presence of enhancing antibodies directed against both type and flavivirus group specific determinants in the homologous antisera.

The phenomenon of immune enhancement has been shown to be a major pathogenetic mechanism in severe dengue infections (Halstead, 1982). Porterfield (1981) has also suggested that it might be a contributory factor to early death in rabies. The role of this phenomenon in the pathogenesis of other infections is far from being understood. It is possible that the present observations on immunological enhancement of Wesselsbron virus in P388D$_1$ cells may have important veterinary and public health implications, if indeed infection enhancement occurs in nature. Although antibodies to flaviviruses have been found in sheep sera in Nigeria (A. H. Fagbami, unpublished), transmission experiments indicated that pre-infection flavivirus antibody was capable of reducing the degree of viraemia and severity of Wesselsbron virus disease in West African

Table 3. Enhancement of Wesselsbron virus* in P388D$_1$ cells by various dilutions of homologous and heterologous antibody

Virus	Fold enhancement ratios at various antibody dilutions				Enhancing antibody titre
	1:50	1:500	1:5,000	1:50,000	
Wesselsbron	×5	×10	×9	×3	≥50,000
Potiskum	×5	×8.5	×2.7	0	≥5,000
West Nile	×5	×1.3	0†	0	≥50
Yellow fever	×3	×2	0	0	500
Zika	×6	×5	0	0	≥500

*Virus tested at multiplicity of infection (MOI) of 0.001.
†0 = no enhancement.

Discussion

Wesselsbron virus has produced severe epizootics in livestock and human infections in Africa (Weiss, Haig & Alexander 1956; Tomori *et al.*, 1981). It has been isolated from mosquitoes in several other West African countries (Annual Report Institute Pasteur, Dakar Senegal, 1979). In man, Wesselsbron virus produces a dengue-like illness, but the disease in sheep in severe and characterized by high mortality in lambs and abortion in pregnant ewes.

Dwarf sheep (Fagbami & Ojeh, 1981). However, an overt Wesselsbron disease had been reported in an individual with pre-infection yellow fever antibody (Tomori *et al.*, 1981). Whether or not that was a case of enhanced Wesselsbron disease was not assessed.

In many parts of Nigeria, Wesselsbron and other flavivirus antibodies are prevalent in the human and animal populations (Fagbami *et al.*, 1977). It is, therefore, possible that this antibody-mediated enhancement of flavivirus infections may be occurring in nature. However, such enhanced infections may be unrecognized, in view of the fact that infection enhancement may not necessarily result in an increase in disease severity but may manifest as acceleration of onset or course of disease, increased viraemia or pathological lesions.

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