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Singh's *Aedes albopictus* Cell Cultures as Helper Cells for the Adaptation of Obodhiang and Kotonkan Viruses of the Rabies Serogroup to Some Vertebrate Cell Cultures

SONJA M. BUCKLEY

Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510

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Multiplication of rabies serogroup viruses, Obodhiang and kotonkan, was induced in vertebrate cell cultures by using Singh's *Aedes albopictus* cell cultures as "helper cells."

Shope et al. (3) established the existence within the rhabdoviruses of a rabies serogroup composed of rabies, Lagos bat, and Mokola (IbAn 27377) viruses. Subsequent studies (R. E. Shope, Rabies virus—antigenic relationships, *in* G. M. Baer, ed., The natural history of rabies, in press) have added two other rhabdoviruses to this serogroup: Obodhiang (SudAr 1273-64), isolated from unengorged *Mansonia uniformis* in the Sudan (2), and kotonkan (IbAr 23380), isolated from *Culicoides* spp. in Ibadan, Nigeria (G. E. Kemp et al., manuscript in preparation). (The name Obodhiang, proposed by J. R. Schmidt, is not yet published, and its use here is not intended to constitute priority.)

In preliminary experiments in this laboratory, mouse-brain preparations of Obodhiang (strain SudAr 1154-64) and kotonkan were tested for their ability to infect cultures of invertebrate and vertebrate cell lines. Both viruses readily infected monolayer cultures of Singh's *Aedes albopictus* cell line (4) grown in 2-oz (ca. 59.125-ml) flint glass prescription bottles and incubated at 30 C; neither virus multiplied in cultures of Singh's *A. aegypti* cell line (4) incubated at 30 C or in cultures of the LLC-MK_2, Vero, and BHK-21 cell lines incubated at 36 C.

Initially, the presence of virus in the mosquito cells was determined by subinoculation of either undiluted fluid phase or combined fluid and cell phases into 2-day-old mice by the intracerebral route. Subsequently it was observed that subinoculation from *A. albopictus* cells infected with either virus repeatedly and consistently induced plaque formation and cytopathic effect (CPE) in Vero cell cultures. These mosquito "helper cells" were then studied with regard to percentage of infected cells and production of intracellular and extracellular virus, by using methods described in detail elsewhere (1).

In Vero cell cultures inoculated with Obodhiang- or kotonkan-infected *A. albopictus* cells at a multiplicity of infection of 1 infant mouse 50% lethal dose per 50 insect cells, plaques were formed only when at least 0.06% (Obodhiang) or 0.05% (kotonkan) of the insect cells were infected (Table 1). Amounts of intracellular virus exceeded amounts of extracellular virus, with plaque-forming intracellular virus demonstrated on postinoculation days 22 (Obodhiang) and 36 (kotonkan) and plaque-forming extracellular virus (both agents) demonstrated on postinoculation day 36.

In a subsequent experiment, inoculation of cell phase (containing at least 0.05 to 0.06% infective centers) of Obodhiang- and kotonkan-infected *A. albopictus* cell cultures regularly induced CPE in both Vero and BHK-21 cell cultures. In these vertebrate cell cultures under fluid medium, multiple focal lesions appeared within the cell sheets at 7 to 10 days postinoculation, the lesions progressing to CPE of 3+ (50-90% of cells destroyed) for kotonkan virus and to CPE of 4+ (90-100% of cell destroyed) for Obodhiang virus. Serial passages of the two viruses were easily established in both Vero and BHK-21 cell lines by subinoculation of combined fluid and cell phases of infected cultures into new cultures at 1- to 2-week intervals. Infectivity titers varied from 3.5 to 6.5 $\log_{10}$ per ml, with the 50% tissue culture dose titers

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TABLE 1. *Percentage of infective centers and production of intracellular and extracellular virus obtained with Obodhiang and kotonkan viruses on primary infection of Aedes albopictus cells, as determined by subinoculation into Vero cells*

Virus	Days after subinoculation into Vero cells	No. *A. albopictus* cells plated on Vero cells (× 100)	No. plaques counted	Infective centers (%)	Amt of virus detected[a]	
					Intracellular	Extracellular
Obodhiang, SudAr 1154-64	8	660	0	0	0	0
	14	1,280	0	0	0	0
	22	50	3	0.06	1,585	0
	36	40	22	0.55	7,080	100
Kotonkan, IbAr 23380	8	1,320	0	0	0	0
	14	1,300	0	0	0	0
	22	2,600	0	0	0	0
	36	60	3	0.05	2,510	80

[a] Expressed as plaque-forming units per milliliter.

obtained generally being higher in the BHK-21 cell line.

Plaque formation in Vero cell cultures and the development of CPE in both Vero and BHK-21 cell cultures under fluid medium were specifically inhibited by Obodhiang and kotonkan hyperimmune mouse ascitic fluids.

Thus, by the use of "helper cells" of mosquito origin, Obodhiang and kotonkan viruses have been adapted to cultures of two vertebrate cell lines. These vertebrate cell cultures, in turn, will serve as potentially useful tools for plaque purification of the viruses, and subsequently for quantitative in vitro assays as well as in vitro purification.

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LITERATURE CITED

1. Libikova, H., and S. M. Buckley. 1971. Studies with Kemerovo virus in Singh's *Aedes* cell lines. Acta Virol. **15**:393–403.
2. Schmidt, J. R., M. C. Williams, M. Lulu, A. Mivule, and E. Mujombe. 1965. Viruses isolated from mosquitoes collected in the southern Sudan and western Ethiopia. East Afr. Virus Res. Inst. Rep. **15**:24.
3. Shope, R. E., F. A. Murphy, A. K. Harrison, O. R. Causey, G. E. Kemp, D. I. H. Simpson, and D. L. Moore. 1970. Two African viruses serologically and morphologically related to rabies virus. J. Virol. **6**:690–692.
4. Singh, K. R. P. 1967. Cell cultures derived from larvae of *Aedes albopictus* (Skuse) and *Aedes aegypti* (L.) Curr. Sci. **36**:506–508.

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