SUSCEPTIBILITY OF *AEDES NOVALBOPICTUS* CELL LINE TO INFECTION WITH SOME ARBOVIRUSES

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ABSTRACT

Susceptibility of *Aedes novalbopictus* cell line to infection with chikungunya (Group-A); West Nile, Japanese encephalitis, dengue types 1, 2, 3 and 4 (Group-B); Chandipura (VSV group) and Ganjam (ungrouped) viruses has been studied. All the above-mentioned 9 arboviruses multiplied without showing any obvious cytopathic effect in this cell line. Detailed studies carried out on the growth of 4 types of dengue viruses indicated that the titre of cell associated virus was higher than that of extracellular virus.

INTRODUCTION

ESTABLISHMENT of several new cell lines from many species of mosquitoes and their susceptibility to infection with some common arboviruses have been reported from this laboratory earlier[1-14]. Recently, new cell lines were established from yet another species of mosquito, *viz.*, *Aedes novalbopictus*[15]. The present communication deals with the studies carried out on the susceptibility of this new cell line to infection with some arboviruses.

MATERIALS AND METHODS

Cell line.—Cells from a continuous line of *A. novalbopictus* (ATC-173) from 41 and 45 passage levels were employed. The details of the maintenance of the cell line and the culture medium were described earlier[15]. *A. albopictus* cells[1] from the line ATC-15 from 19 to 30 passage levels were employed to assay some of the viruses.

Viruses.—The following 9 common arboviruses representing the major serogroups were tested.

Group A : Chikungunya (CHIK), VRC No. 634029, mouse passage 12.

Group B : West Nile (WN), VRC No. G 22886, mouse passage 17.

Japanese encephalitis (JE), VRC No. P 20778, mouse passage 10.

Dengue type 1 (DEN-1), VRC No. 703311, 6 passages in *Aedes albopictus* cell culture.

Dengue type 2 (DEN-2), VRC No. 68883, 4 passages in *Aedes albopictus* cell culture.

Dengue type 3 (DEN-3), VRC No. 703539, 6 passages in *Aedes albopictus* cell culture.

Dengue type 4 (DEN 4), VRC No. 684996, 4 passages in *Aedes albopictus* cell culture.

VSV Group : Chandipura (CHP), VRC No. 653514, mouse passage 20.

Ungrouped : Ganjam (GAN), VRC No. G 619, mouse passage 5.

Virus inoculation.—The techniques employed to study the multiplication of the viruses were essentially the same as described by Singh and Paul[2]. Briefly, batches of 20 monolayer culture tubes were inoculated with 0·1 ml of virus suspension as to give 3 to 4 dex $TCID_{50}$ or LD_{50} of the virus per culture tube. The inoculum were simultaneously titrated either in mice or in tissue culture as to determine the exact dose of virus inoculated. After 2 hours absorption, infected cell sheets were washed twice with Rinaldini's salt solution and fed with fresh medium. In order to study the growth of 4 types of dengue viruses in detail, extracellular and cell associated viruses were harvested separately from 2 infected culture tubes at '0' hour and on post-inoculation (PI) days 3, 6, 10 and 15. Whereas, in case of CHIK, WN, JE, CHP and GAN viruses, they were harvested only on the 10th PI day just to test whether *A. novalbopictus* cells supported their multiplication. Batches of tubes containing 0·5 ml medium without cells, inoculated with 0·1 ml virus suspension, were used as controls.

Virus assay.—All the four types of dengue viruses were assayed in normal *A. albopictus* cell culture. Whereas, other viruses were assayed in infant (2 to 3-day-old) mice by intracerebral route. The titres were expressed as dex $TCID_{50}$ for tissue culture or LD_{50} for mice. Identity of the viruses from the harvested culture fluids was confirmed serologically in complement fixation test.

RESULTS

The results indicated that all the nine arboviruses tested multiplied in *A. novalbopictus* cells (Table I), without showing any obvious cytopathic effect (CPE). Approximately 100 to 100,000 fold increase in the virus concentration was observed during the first 10 days with these viruses.

The growth curve studies with 4 typ of dengue viruses in *A. novalbopictus* cells (Fig. 1) indicated

that the concentration of cell associated virus was higher than the virus in the extracellular fluid. The difference was generally 2 dex or less. Among the 4 types of dengue virus, type 2 showed approximately 10,000 fold increase, whereas, the others showed approximately 1,000 fold increase during the 15 days of observation.

10,000 fold increase, whereas, CHP showed 1,000 fold increase.

Discussion

The multiplication of nine arboviruses in *A. novalbopictus* cells was comparable to that in *A. albopictus* cells as studied earlier[2,3]. While

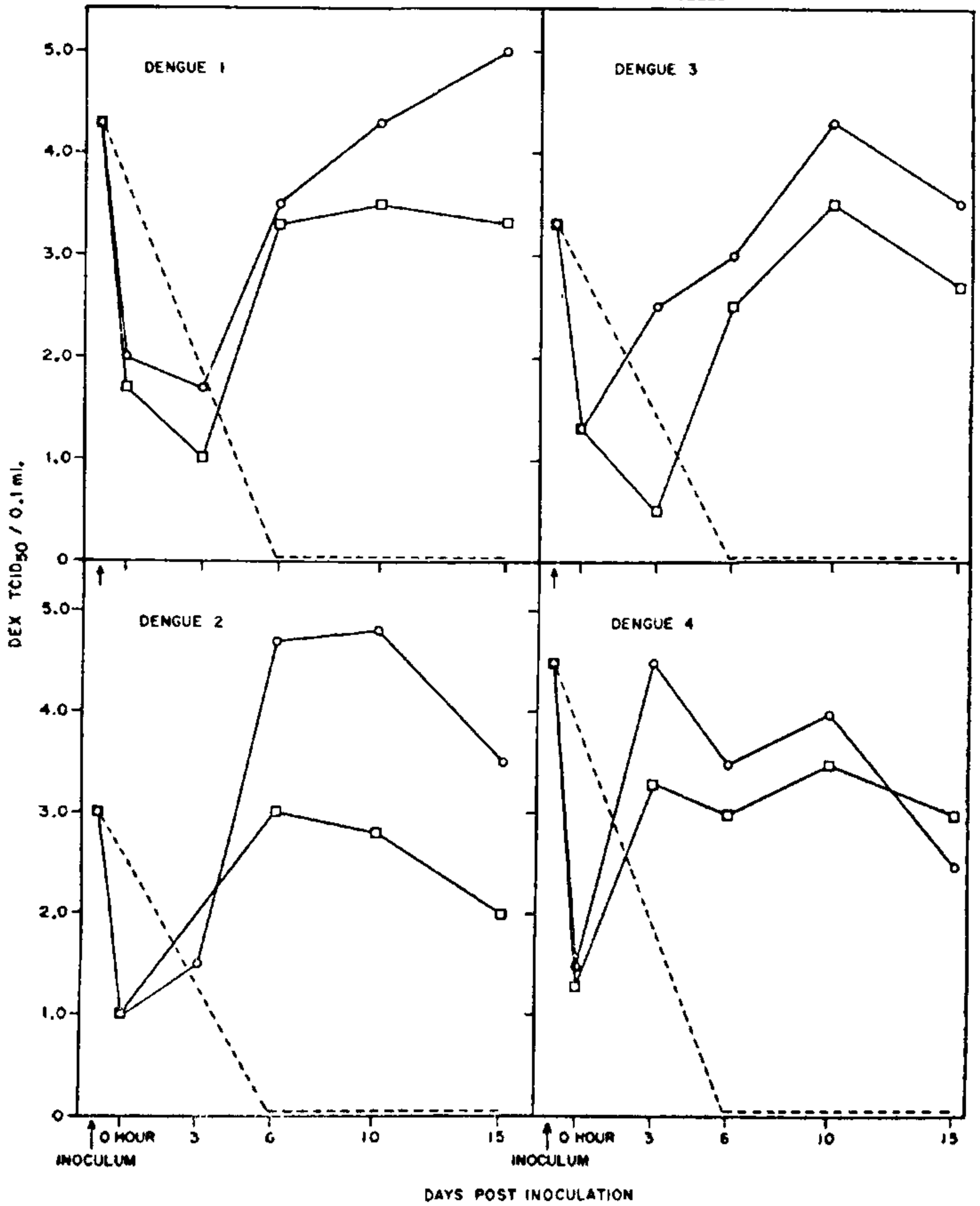

FIG. 1.

Among other viruses, when tested on the 10th PI day WN virus showed the maximum increase (approximately 100,000 fold), whereas, JE virus showed the minimum increase (approximately 100 fold), CHIK and GAN viruses showed approximately

A. albopictus cells showed CPE with group B mosquito borne arboviruses, *viz.*, WN, JE, DEN-1, 2, 3 and 4, no obvious CPE was detected in *A. novalbopictus* cells. Studies carried out on the susceptibility of *A. albopictus* cells to infection with

TABLE I

Multiplication of some arboviruses in Aedes novalbopictus *cell line*

Virus	Titre					
	Inoculum	0 Hour	PI Days			
			3	6	10	15
1. Chikungynya*	2·5	NT	NT	NT	6·5	NT
2. West Nile*	1·5	NT	NT	NT	6·0	NT
3. Japanese encephalitis*	2·5	NT	NT	NT	4·0	NT
4. Dengue type 1**	4·3	2·0	1·7	3·5	4·3	5·0
5. Dengue type 2**	3·0	1·0	1·5	4·7	4·8	3·5
6. Dengue type 3**	3·3	1·0	2·5	3·0	4·3	3·5
7. Dengue type 4**	4·5	1·5	4·5	3·5	4·1	3·1
8. Chandipura*	3·5	NT	NT	NT	6·5	NT
9. Ganjam*	2·5	NT	NT	NT	6·5	NT

* Virus titre, dex LD_{50}/0·02 ml.
**Virus titre, dex $TCID_{50}$/0·1 ml, cell assocciated virus.
NT=Not tested.

4 types of dengue viruses (Guru and Bhat, unpublished data) indicated that yield of these viruses was higher in *A. albopictus* cells than in *A. novalbopictus* cells. It is thus evident that *A. albopictus* cells are more susceptible to arbovirus infection than *A. novalbopictus* cells.

It is interesting to note that in *A. novalbopictus* cells infected with 4 types of dengue viruses, the concentration of cell assocciated virus was always higher than that present in the extracellular fluid. Similar results have been obtained with these viruses in *A. albopictus* cells (S. N. Ghosh, personal communication).

Earlier studies carried out in this laboratory on the susceptibility of some of the mosquito cell lines to infection with arboviruses indicated that *A. aegypti* cells supported the multiplication of 3 out of $9^{2,3}$, *A. w-albus* 3 out of 6^{14}, and *A. vittatus* 4 out of 6^{13} viruses tested. Thus it is evident that *A. novalbopictus* cells are more susceptible to arbovirus infection than *A. aegypti*, *A. w-albus* and *A. vittatus* cells.

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1. Singh, K. R. P., *Curri Sci.*, 1967, 36, 506.
2. — and Paul, S. D., *Ibid.*, 1968, 37, 65.
3. — and —, *Indian J. Med. Res.*, 1968, 56, 815.
4. Banerjee, K. and Singh, K. R. P., *Ibid.*, 1968, 56, 812.
5. — and —, *Ibid.*, 1969, 57, 1003.
6. Paul, S. D. and Singh, K. R. P., *Curr. Sci.*, 1969, 38, 241.
7. —, — and Bhat, U. K. M., *Indian J. Med Res.*, 1969, 57, 339.
8. Pavri, K. M. and Ghosh, S. N., *Bull. Wld. Hlth. Org.*, 1969, 40, 984.
9. Singh, K. R. P. and Paul, S. D., *Ibid.*, 1969, 40, 982.
10. Bhat, U. K. M. and Singh, K. R. P., *Curr. Sci.*, 1970, 39, 388.
11. Singh, K. R. P. and Bhat, U. K. M., *Experientia*, 1971, 27, 142.
12. —, — and Paul, S. D., *Indian J: Med: Res.*, 1971, 59, 31.
13. Bhat, U. K. M. and Goverdhan, M. K., *Curr. Sci.*, 1972, 41, 480.
14. Singh, K. R. P., Goverdhan, M. K. and Bhat, U. K. M., *Indian J. Med. Res.*, 1973, 61, 1134.
15. Bhat, U. K. M. and Guru, P. Y., *Exp. Parasitol.*, 1973, 33, 105.