AD NUMBER

AD860406

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AUTHORITY

Army Biological Defense Research Lab ltr dtd 29 Sep 1971

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TECHNICAL MANUSCRIPT 553

CHIKUNGUNYA VIRUS INFECTION OF CELL MONOLAYERS BY CELL-TO-CELL AND EXTRACELLULAR TRANSMISSION

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W. Douglas Zimmerman

SEPTEMBER 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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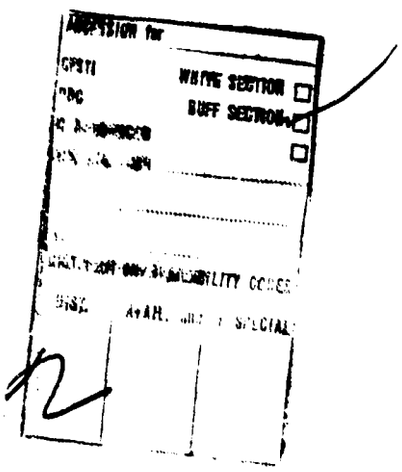

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Experimental Aerobiology Division
AEROBIOLOGY & EVALUATION LABORATORIES

Project 1B562602A059

September 1969

CHIKUNGUNYA VIRUS INFECTION OF CELL MONOLAYERS BY CELL-TO-CELL AND EXTRACELLULAR TRANSMISSION*

ABSTRACT

Both cell-to-cell and extracellular transmission of chikungunya virus infection was demonstrated in BHK21/C13 cell monolayers. The mode of virus infection may depend on the cell line.

A preliminary study directed toward the development of an immunofluorescent assay of chikungunya virus based on the enumeration of individual infected cells in monolayer cultures was hampered by the appearance of foci containing several infected cells. These foci were noted even when inoculated cell monolayers were incubated in the presence of a potent antiviral serum overlay. That virus infection may proceed by cell-to-cell transmission was suggested by these observations. This mode of infection in cell cultures has been reported previously with herpes-B[1] and respiratory syncytial viruses.[2] In this study, we offer evidence that both cell-to-cell and extracellular transmission of chikungunya virus infection occurs in cell monolayers.

The Banganike strain of chikungunya virus,[3] supplied by Dr. William A. Hankins, Fort Detrick, Frederick, Maryland, was used. It was in the form of a 10% suckling mouse brain suspension and had a titer of $10^{8.9}$ LD_{50} per ml by intracerebral assay in this host. The two baby hamster kidney (BHK) cell lines used in this study, designated BHK21/C13 and BHK21, were obtained from the American Type Culture Collection, Rockville, Md., and from Microbiological Associates, Bethesda, Md., respectively. The BHK21/C13 line morphologically consists of elongated fibroblastic cells; the BHK21 line is mainly short fibroblastic cells. Nutrient medium for both cell lines consisted of Earle's minimum essential medium (MEM) supplemented with 1% glutamine (200 mM), 10% tryptose phosphate broth, and 10% fetal calf serum (FCS). Maintenance medium consisted of equal parts of nutrient medium and MEM. Nutrient and maintenance media for L-929 cells were medium 199 plus 5% FCS. Nutrient medium for guinea pig lung cells was Eagle's basal medium (BME), 10% FCS, and 0.5% lactalbumin hydrolysate; cells were maintained in BME plus 5% FCS. All media contained 50 μg of streptomycin and 75 μg of kanamycin per ml. Infected cell monolayers were stained by the direct immunofluorescent method, using hyperimmune rhesus monkey serum conjugated with fluorescein isothiocyanate[4]

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to observe the spread of virus and to enumerate foci of infection. Reagents, fluorescent equipment, and other technical procedures used to carry out the immunofluorescent method are described in detail elsewhere.[5]

To substantiate the preliminary indication of cell-to-cell transmission of chikungunya virus infection in BHK21/C13 cell monolayers, cover slip cell cultures were inoculated with 0.2 ml of an appropriate dilution of virus suspension and inoculum was attached as described previously with another arbovirus.[5] Cover slip cell cultures were then incubated at 35 C for 1 hour in the presence of maintenance medium to allow for virus penetration. A group of these inoculated cell monolayers was then incubated at 35 C for 20 hours in the presence of a 1/10 dilution of antiviral serum in maintenance medium. Another group of inoculated cell monolayers was dispersed with trypsin and suspended in maintenance medium containing antiviral serum. The cells were then sedimented onto uninfected cell monolayers with the aid of centrifugal force (500 x g, 10 min). The residual inoculum, free of cells, was pooled, and introduced onto uninfected cell monolayers. All cell cultures were then incubated at 35 C for 20 hours and subsequently stained. Results (Table 1) show that foci of immunofluorescent cells appeared in the presence of antiviral serum and, also, with trypsin-dispersed cells that had been sedimented onto uninfected cell monolayers (Fig. 1). Recovery of infected cells dispersed with trypsin was 80% efficient. No infectious virus was detected in cell-free inoculum. The ability of cell-associated virus (trypsin-dispersed cells) to initiate foci of infected cells in the presence of antiviral serum is direct evidence of cell-to-cell transfer of virus infection.

TABLE 1. EVIDENCE OF CELL-TO-CELL TRANSMISSION OF CHIKUNGUNYA VIRUS INFECTION IN BHK21/C13 CELL MONOLAYERS

Test Condition	IFU[a] per 50 Microscopic Fields
Virus-inoculated cell monolayers[b]	21
Trypsinized infected cell monolayers, suspended in antiviral serum medium, inoculated onto uninfected cell monolayers	17
Supernatant medium from above, free of cells, inoculated onto uninfected cell monolayers	0

a. Immunofluorescent focus units.
b. Incubated at 35 C for 1 hour to ensure virus penetration into cells, then incubated at 35 C for 20 hours in presence of antiviral serum medium.

To determine whether chikungunya virus infection by cell-to-cell transfer is limited to the BHK21/C13 cell line, cover slip cell cultures of the two BHK and other cell lines were infected with virus and incubated at 35 C for 20 hours either in the presence of medium containing antiviral serum or in maintenance medium. Results (Table 2) show that foci of infected cells occurred only in the two BHK cell lines in the presence of antiviral serum. Large numbers of both foci and individual infected cells were noted in the absence of antiviral serum, which indicates that extracellular infection had occurred. The detection of virus in harvested medium from these latter cell cultures is additional evidence for extracellular transfer of infection. No virus was found in medium harvested from cell cultures incubated earlier with antiviral serum. In contrast, only individual infected cells were noted in the L-929 and guinea pig lung cell cultures that had been incubated with antiviral serum (Fig. 2). This indicates an extracellular mode of virus infection. Of the cell lines tested, the BHK21/C13 cells appeared to be the most susceptible to chikungunya virus infection. The findings of this study show that chikungunya virus infection of BHK21 cell monolayers may take place by both cell-to-cell and extracellular transmission and that the mode of virus transfer may depend on the cell line. In comparable studies with Venezuelan equine encephalomyelitis virus using the BHK21/C13 cell line and other cell lines (L-929, McCoy, guinea pig lung), similar results were obtained.* The observations reported here, together with those on the neoplastic transformation of the BHK cell line and its derivatives by viruses,[6] suggest that these cells possess some unique biological membrane structure or physiology that is conducive for cell-to-cell transmission of virus particles.

TABLE 2. CELL-TO-CELL AND EXTRACELLULAR TRANSMISSION OF CHIKUNGUNYA VIRUS INFECTION IN DIFFERENT CELL MONOLAYERS

Cell Line	Antiviral Serum Medium	Cell Maintenance Medium
BHK21/C13	2.4×10^8 IFU[a]/ml	TNTC[b]
BHK21	2.6×10^7 IFU/ml	TNTC
L-929	5.8×10^5 CIU[c]/ml	5.3×10^5 IFU/ml
Guinea pig lung	2.1×10^5 CIU/ml	1.5×10^5 IFU/ml

a. Immunofluorescent focus units consisting of 3 to 15 infected cells.
b. Too numerous to count; cell monolayers contained both fluorescent foci and individual infected cells.
c. Cell-infecting units; individual infected cells.

* Unpublished data.

FIGURE 1. Fluorescent Focus of Chikungunya Virus Infection in BHK21 Cell Monolayer in the Presence of Antiviral Serum. 225X

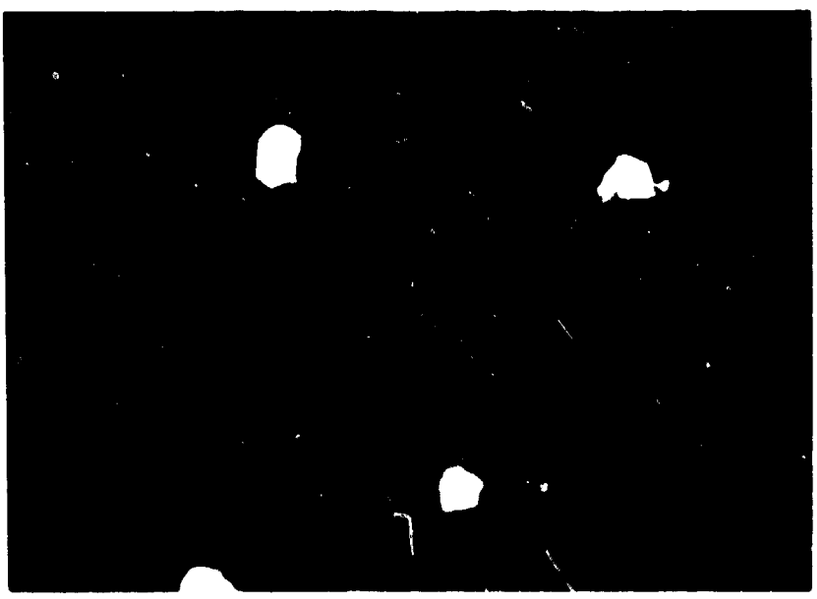

FIGURE 2. Individual Guinea Pig Cells Infected with Chikungunya Virus in the Presence of Antiviral Serum. 225X

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701	Unclassified 2b. GROUP

3. REPORT TITLE

CHIKUNGUNYA VIRUS INFECTION OF CELL MONOLAYERS BY CELL-TO-CELL AND EXTRACELLULAR TRANSMISSION

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)

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W. Douglas Zimmerman

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
September 1969	11	6

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B562602A059	Technical Manuscript 553
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) CMs 6584
d.	

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Department of the Army Fort Detrick, Frederick, Maryland, 21701

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14. Key Words

Chikungunya virus
Cell monolayers
Infection transmission
Immunofluorescent assay
FITC

DD FORM 1 NOV 68 1473

Unclassified
Security Classification