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REPRODUCTION OF LASSA VIRUS IN DIFFERENT CELL CULTURES

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Summary. — Sierra Leone strain of Lassa virus was growing to high titres of 10^5—10^6 plaque forming units (PFU) per ml in Vero, L and swine kidney cell lines as well as in diploid human cells and primary human embryo kidney cells. As many as 80% of the cells became infected as demonstrated by the immunofluorescence (IF) technique. In BHK-21, CV-1, HeLa, FL, HEp-2 and dog kidney cell lines, the virus reproduced to lower titres (10^4—10^5 PFU per ml), whereas in primary chick embryo fibroblasts it did not multiply at all. The virus formed plaques under agar overlay only in CV-1 and Vero cells.

Key words: *Lassa virus*; *reproduction*; *cell cultures*

Introduction

Vero cells are commonly used for the isolation and investigation of Lassa virus. There is only one available information concerning the ability of Lassa virus to multiply in other cell lines as proved by induction of cytopathic effect (CPE) and by presence of virus antigen on days 5 and 14 post infection (p.i.) demonstrated using IF technique (van der Groen *et al.*, 1978). In the present paper we investigated the kinetics of infectious virus accumulation and the occurrence of viral antigens in different cells including primary, continuous and diploid cell cultures. The plaque-forming ability of the virus in these cell cultures was studied as well.

Materials and Methods

Lassa virus strain Sierra Leone was obtained from the Collection of Viruses, the Ivanovsky Institute of Virology, Moscow. The virus cultivation, plaquing, titration and determination of viral antigens by IF were described previously (Lukashevich *et al.*, 1981*a*, *b*: 1982).

Cell cultures. Besides continuous cell lines, namely human epithelial cells (HeLa, FL and HEp-2), green monkey kidney cells (Vero and CV-1), hamster kidney cells (BHK-21), mouse fibroblast (L cells), swine kidney cells (SPEV), dog kidney cells (MDCK), also primary chick embryo fibroblast(CEF) and human embryo kidney (HEK) cell cultures as well as diploid human embryo lung (HELD) cells were employed. Cell culture monolayers grown in flasks were exposed to the virus at multiplicity of infection (MOI) 0.1 PFU/cell. After 90 min adsorbtion at 37 °C, the virus was removed, cells were washed and maintenance medium was added. In some experiments, non-adsorbed virus was neutralized by addition of immune serum for 30 min (37 °C). The virus-infected cells were incubated at 37 °C for 72 hr. At 12 hr intervals the medium was removed from two flasks to determine the virus yield: the cells were trypsinized and cell smears were prepared for IF examination. In several cases infected cells grown on cover slips were examined by IF technique.

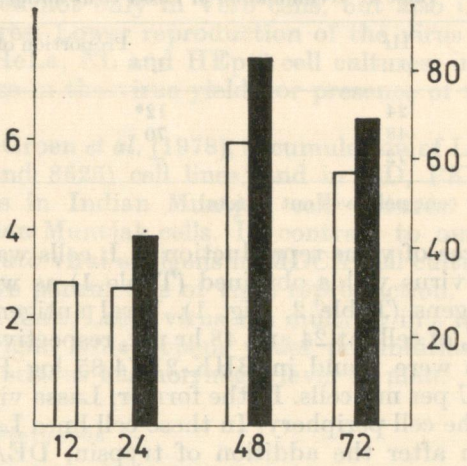

Fig. 1.
Kinetics of Lassa virus reproduction in Vero cells
The MOI was 0.1 PFU/cell: the virus yield was determined in cultivation medium (empty columns); the proportion of cells showing virus antigens was counted (black columns).
Abscissa: hr p.i.; left ordinate: log PFU per ml; right ordinate: percentage of cells containing viral antigens.

Results and Discussion

The first experiments were aimed at the characterization of acute Lassa virus infection in Vero cells. As shown in Fig. 1, when MOI of 0.1 PFU was used, the maximum virus yield in medium fluid was obtained between 48 and 60 hr p.i. later on followed by a decrease in virus titre. As determined by IF, Lassa virus antigens were found in cytoplasm of Vero cells after 24 hr p.i. as granules of varying shape and size (Fig. 2). Within an interval of 48—60 hr p.i., viral antigens were determined in more than 80% of cells. The assay of Lassa virus in Vero cells by slightly modified plaque technique of Porterfield and Allison (1960) resulted in an appearance of small plaques (diameter of 0.3—0.5 mm) on day 4 p.i.

In further experiments, the ability of Lassa virus reproduction was investigated in BHK-21, L and MDCK cell lines, which are known to be permissive for other arenaviruses (Pfau *et al.*, 1973; Martinez Segovia *et al.*, 1974; Gangemi *et al.*, 1977; Gard *et al.*, 1977; Dutko and Pfau, 1978). The

Table 1. The kinetics of Lassa virus reproduction in different cell cultures

Hr p.i.	Virus titre* in the cultivation medium										
	BHK-21	MDCK	L	CV-1	HeLa	FL	SPEV	HEP-2	CEF	HEK	HELD
12	2.15	2.54	3.53	0	2.62	2.95	3.81	3.45	2.97	3.61	4.03
24	2.88	3.11	3.83	2.81	3.65	3.65	4.08	4.00	3.86	3.96	4.51
36	3.57	3.84	5.04	3.89	3.92	4.60	5.04	4.85	2.90	5.08	5.90
48	3.71	4.04	5.18	3.68	4.52	4.78	5.64	5.04	2.18	5.89	5.70
60	4.85	3.93	5.94	5.45	4.59	4.96	5.45	4.08	2.24	6.26	5.45
72	4.67	3.63	5.18	3.87	4.81	4.90	5.20	4.79	0	5.98	5.18

* log PFU/ml, SPEV = swine kidney cells, MDCK = dog kidney cells, CEF = chick embryo fibroblasts, HEK = human embryo kidney cells (primary), HELD = human embryo lung cells (diploid).

Table 2. Accumulation of Lassa virus antigen in L, HEK and HELD cell cultures

Hr p.i.	Proportion of cells revealing virus antigens		
	L	HEK	HELD
24	12*	15	21
48	70	52	81
72	—	64	63

* per cent positives out of total

degree of virus reproduction in L cells was similar to that in Vero cells as to the virus yields obtained (Table 1) as well as to the accumulation of viral antigens (Table 2, Fig. 1). Viral antigens were demonstrated in 12% and 70% of cells at 24 and 48 hr p.i., respectively. Virus yields lower than in Vero cells were found in BHK-21 (4.85 log PFU per ml) and MDCK (4.04 log PFU per ml) cells. In the former, Lassa virus antigen was only slightly visible at the cell periphery. In these cell lines Lassa virus did not form any plaques even after the addition of trypsin, DEAE-dextran or protamine sulphate to the agar overlay.

The further group of cell cultures used was represented by CV-1, HeLa, FL, SPEV and HEp-2 cell lines. Lower reproduction of Lassa virus (the maximum titres being 3.87—3.89 log PFU units per ml) was found in CV-1 than in Vero cells, though both cell lines are of the same origin (green monkey). At the same time, the virus formed plaques also in CV-1 cell cultures. On the other hand, higher virus reproduction in HeLa, FL, HEp-2 (to 5 log PFU per ml) and SPEV (to 6 log PFU per ml) cells was not accompanied by plaque formation in these cell lines. In HEp-2 cells the viral antigen was revealed in the form of single marked grains in the cytoplasm.

The last group of cells studied consisted of primary CEF and HEK cultures and HELD cells. In CEF cultures the Lassa virus did not multiply at all. In HEK and HELD cells virus titres as high as in Vero and L cells were found (Table 1). The high degree of virus reproduction in these cells was confirmed also by the kinetics of viral antigen accumulation (Table 2). In HELD cells the viral antigens were seen as intensively stained granules at the cytoplasm periphery. Again, in these cell cultures the virus did not cause any plaque formation in these cell cultures. Though in Vero cells Lassa virus caused CPD on days 5—6 p.i. when using low MOI, in remaining cell cultures no CPE was observed.

According to the reproduction of Lassa virus the 12 different cell cultures could be divided into several groups as follows: a) Vero cells and cell lines previously used in other experiments with arenaviruses (BHK-21, L, MDCK); b) cell lines, which either were not or were rarely employed in arenavirus studies (CV-1, HeLa, FL, SPEV, HEp-2) c) and finally, primary (CEF and HEK) and diploid (HELD) cell cultures. In all cell cultures under study, the virus reproduction was evaluated by the kinetics of virus yield in cultivation medium. In addition, 1—2 cell cultures from each group were examined for the presence of viral antigens. At the same time, the ability of Lassa virus to cause CPE and form plaques was investigated in all cell cultures.

Lassa virus reproduced fairly well not only in Vero cells, but also in L, SPEV HEK and HELD cell cultures. Lower reproduction of the virus was found in BHK-21, MDCK, CV-1, HeLa, FL and HEp-2 cell cultures; in infected CEF cultures neither increase in the virus yield nor presence of viral antigens were demonstrated.

According to the data of van der Groen *et al.* (1978), accumulation of Lassa virus was found in viper (VSW and 8625) cell lines, and in RD, PK 15, BHK-21 and Ptk-2 cells as well as in Indian Muntjak cell cultures. The virus caused CPE in Vero and Indian Muntjak cells. In contrast to our results, the authors did not demonstrate viral antigens in MDCK cell cultures. However, they used only qualitative parameters of virus reproduction.

As follows from above mentioned data, Lassa virus can multiply in a number of cell cultures of different origin, which opens wider possibilities for investigation of this agent causing serious haemorrhagic fever in man.

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Explanation of Micrographs (Plate XXXII);

Fig. 2. Lassa virus antigens in infected Vero cells. The virus-infected cells (MOI = 0.1 PFU) were cultivated for 72 hr at 37 °C, trypsinized and the smears of the cell suspension were fixed in acetone and examined by indirect IF as described (Lukashevich *et al.*, 1981*b*).

Fig. 3. Control, non-infected Vero cells.