APPLIED MICROBIOLOGY, May 1970, p. 791-794

Vol. 19, No. 5
Printed in U.S.A.

Viremia and Virus Measurements of Rabbit Pox in CV-1 Cells

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Received for publication 3 February 1970

Rabbit pox virus (RPV) produced cytopathic effect (CPE) in five types of cells grown in tissue cultures. The CPE on CV-1 cells was characterized by cell fusion and lysis. The CV-1 line is a useful and sensitive cell culture for measuring concentrations of RPV in blood and tissues of infected rabbits. Viremia was detected between the 2nd and 4th days after parenteral infection. By the 6th and 7th days, the concentration of RPV in various tissues ranged from $10^{5.3}$ to $10^{8.5}$ $TCID_{50}$/g. Cross-reactivity was demonstrated by the fluorescent-antibody technique between rabbit pox, vaccinia, and monkey pox viruses.

Rabbit pox is a highly infectious (2, 5, 6, 8) and fatal disease of rabbits. The disease shares many clinical and pathological features of ectromelia (4), monkey pox (9–11), and smallpox (3). While restudying the ways that rabbit pox virus (RPV) is spread, we found the CV-1 line of culture cells to provide a sensitive method for delineating and measuring viremia and the "load" of virus in various body tissues.

MATERIALS AND METHODS

Stock of virus. The Utrecht strain of RPV obtained from the American Type Culture Collection (VR-157) was passed once in primary bovine embryo kidney cells (BEK). The titer of the stock was $10^{6.0}$ $TCID_{50}$/ml in BEK cells.

Tissue cultures. Various cell cultures were studied, including primary cultures of rhesus monkey kidney, human amnion, and human thyroid; several continuous cell cultures, namely BEK and green African monkey kidney (CV-1) cells, were used also. Cells were grown to form monolayers in glass tubes or in plastic petri dishes (10). CV-1 cells were used exclusively for the titration of blood and tissue suspensions. Cells grown in glass tubes were rotated mechanically; cells grown in plastic petri dishes were placed in an atmosphere of 5% CO_2. The temperature of incubation was 37 ± 0.5 C.

Virus titration. Infectivity end points of the stock virus were based on cytopathic effect (CPE) in monolayer cell cultures (Table 1). Lysed whole blood and 10% tissue suspensions were serially diluted (10-fold) and titrated in CV-1 cells. Methods of handling and preparing blood and tissues for virus isolation were similar to those recounted in earlier reports (11).

Rabbits. New Zealand, white 3- to 4-month-old rabbits weighing about 1.5 kg were used. Rabbits were inoculated with RPV either by intravenous (IV), intramuscular (IM), intradermal (ID), intratesticular, or oral routes. Clinical illnesses and temperatures were recorded. Procedures for collection of blood and tissues were identical to earlier studies (11).

RESULTS

CPE on cell cultures. All five types of cell cultures used were destroyed by RPV. The time of onset of CPE and progression to cell destruction are given in Table 1. Infectivity titers ranged from $10^{4.7}$ to $10^{6.4}$ $TCID_{50}$/ml. Cells derived from simian and bovine species provided titer values about 10-fold ($\geq$0.7) greater than human cells. With the exception of CV-1 cells, the earliest changes consisted of cellular rounding, contact separation, cell fusion, and detachment from glass.

Development of CPE on CV-1 cells was characterized by cell fusion and formation of multinucleated giant cells (Fig. 1). Within 6 to 12 hr, huge cellular syncytia formed with subsequent rapid lysis of cells. In this respect, CPE or RPV differed strikingly from that of vaccinia or monkey pox virus; these latter two viruses do not form syncytia on CV-1 cells. The main features relate to cellular granulation, cell retraction, and rounding followed by cell lysis. The time required for the appearance of CPE and completion of CPE (4+) was relatively short. The most sensitive cell culture to RPV was shown to be the CV-1 line in which $10^{4.0}$ $TCID_{50}$ of virus gave a 4+ CPE

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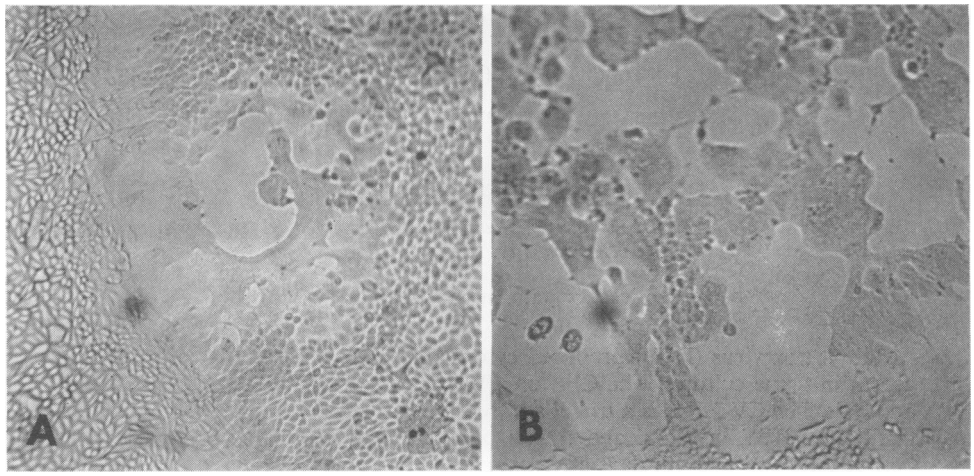

FIG. 1. *Cytopathic effect (CPE) of rabbit pox virus in CV-1 cells observed at 48 hr after infection. (A) Inoculation of a 10^{-5} dilution of the virus shows an early CPE with a syncytium. (B) Inoculation of a 10^{-2} dilution of the virus represents a complete CPE with multinucleated giant cells. × 60.*

within 2 days, in contrast to 4 days for other cell cultures. RPV plaques can easily be produced in CV-1 cells grown in petri dishes overlaid with agar. Plaque titers and tube titers (CPE) were equivalent.

Pathogenicity and virulence. As reported by others, rabbit pox is an extremely virulent disease of rabbits. Eight rabbits were inoculated by various routes with $\sim 10^{5.0}$ $TCID_{50}$ of virus (Table 2); all eight rabbits died 6 to 8 days later.

The clinical events and the pathological findings corresponded closely to those noted in earlier reports (5, 6, 8). No attempt will be made here to describe them in detail. Briefly, the infected rabbits developed fever, generalized papular cutaneous eruption, and dehydration; these symptoms were followed soon afterwards by hypothermia and death of animals. Other signs, namely, diarrhea, nasal and eye discharges, and lymphadenopathy, were observed in some rabbits. The incubation period varied between 2 and 4 days. Rabbits usually died within 1 to 4 days after the onset of rash. At necropsy the spleen and liver contained many scattered small gray-white nodules; histologically these lesions represented areas of cellular necrosis surrounded by zones of mononuclear cells. Additionally, diffuse inflammatory infiltrates, edema, hemorrhage, vasculitis, and necrosis were found in skin, lymph nodes, lungs, kidneys, and testes.

Viremia. RPV was found in the blood of eight rabbits (Table 2). The concentration of the virus in blood ranged from $10^{1.7}$ to $10^{5.5}$ $TCID_{50}$/ml. Rabbits inoculated IV developed viremia by the 2nd day; viremia persisted during the 24-hr interval preceding onset of clinical symptoms and continued probably until death. In rabbits given RPV by IM or ID routes, viremia was delayed, occurring about 48 hr later than after IV inoculation; in these rabbits, viremia was detected by about the 4th day after infection, at the time of, or just before, the appearance of skin rash. Animals died within 1 or 2 days after onset of viremia. The concentration of virus recovered in blood varied greatly between rabbits entered in the several groups.

TABLE 1. *Cell spectrum and sensitivity of rabbit pox virus*

Cell cultures[a]	Cytopathic effect		Virus titer (log_{10} $TCID_{50}$/ml)
	Onset (day)	Completed (day)	
Primary cells			
RMK	2	4	6.3
HA	2	4	5.7
HT	2	4	4.7
Continuous cells			
BEK	1	4	6.0
CV-1	≤1	2	6.4

[a] RMK, rhesus monkey kidney; HA, human amnion; HT, human thyroid; BEK, bovine embryonic kidney; CV-1, secondary monkey kidney.

Recovery of RPV from tissues. RPV was isolated from tissues of four rabbits infected by different routes. A high concentration of RPV was found in all tissues tested (Table 3). At the time of death, no significant differences were discerni-

TABLE 2. *Progression of viremia and onset of symptoms in rabbit pox virus-infected rabbits*[a]

Rabbit no.	Virus inoculation		Log10 TCID50/ml of blood on day after infection							Onset of symptoms (day after infection)	
	Route	Ml	1	2	3	4	6	7	8	Fever	Rash
90	IV	1.0	0	2.7	2.7	3.8	—			3	4
91			0	2.3	3.0	3.3	—			3	5
92	IM	1.0	0	0	0	1.7	—			3	4
93			0	0	0	1.7	ND	—		2	4
94	ID	0.2	0	0	0	2.3	—			3	5
95			0	0	0	3.3	ND	—		2	5
96	S	0.4	0	0	0	1.7	ND	4.3	—	3	4
97			0	0	0	5.5	ND	—		3	4

[a] IV, intravenous; IM, intramuscular; ID, intradermal; S, scarification of skin; —, animal died on indicated day; ND, not done.

TABLE 3. *Recovery of virus from rabbit pox virus-infected rabbits tissues*[a]

Tissues	Log10 TCID50/g of tissue for specified rabbit			
	90[b]	92[c]	94[d]	97[e]
Skin	7.3	8.8	8.5	7.5
Axillary lymph node, right	7.0	ND[f]	8.5	6.8
Axillary lymph node, left	6.5	ND	6.8	6.3
Inguinal lymph node, right	7.0	6.5	5.8	6.8
Inguinal lymph node, left	6.8	7.3	7.5	6.5
Spleen	7.3	6.3	6.0	7.7
Liver	5.3	5.0	5.8	6.9
Kidney	6.5	5.3	ND	ND
Lung	7.5	7.3	7.5	ND

[a] The titer of RPV in testes of rabbits inoculated therein was $10^{7.4}$ $TCID_{50}$/g (range in two rabbits, $10^{7.3}$ to $10^{7.5}$). After water deprivation, four rabbits drank freely of water containing ~10 $TCID_{50}$ of RPV/ml. Two of the four rabbits developed fatal rabbit pox.

[b] Intravenous route of inoculation; death on day 6; concentration of virus in blood not determined on day of death.

[c] Intramuscular route of inoculation; death on day 6; concentration of virus in blood not determined on day of death.

[d] Intradermal route of inoculation; death on day 6; concentration of virus in blood not determined on day of death.

[e] Inoculation by scarification of skin; death on day 7; concentration of virus in blood not determined on day of death.

[f] Not done.

ble in the pattern of virus distribution in tissues of rabbits infected by different portals (IV, IM, and ID). Very probably at the time of death virus multiplication was maximal; high concentrations of RPV were associated with extensive cellular necrosis.

Antigen-antibody reaction. An effort was made to demonstrate RPV in infected tissues by the direct fluorescent-antibody method. Specific fluorescence was seen in the tissues of spleen and liver by using either vaccinia or monkey pox antiserum conjugates (7, 9). Unfortunately, sufficient amounts of anti-RPV serum were not available for comparative studies of RPV, vaccinia virus, and monkey pox virus. RPV neutralizing antibody was easily demonstrated by a plaque reduction test; convalescent serum of rabbit 129 had an 80% plaque reduction at a serum dilution of 1:128.

DISCUSSION

CV-1 cells are sensitive to RPV, producing a kind of CPE remarkably different from that of other cell cultures used in our study. The morphological differences in CPE produced by RPV on CV-1 cells are also strikingly different from the CPE of vaccinia and monkey pox viruses, at least for those strains we have studied. The early onset of cellular injury with large syncytia, early detachment from glass, and possibly the progression of cytological change resembles in part changes described by Appleyard et al. (1) for RPV on ERK (HeLa) cells.

Our results indicate that the CV-1 cell culture may be as sensitive as the choriallantoic membrane of the chick embryo is for detecting RPV; our titer values were equal to or exceeded those

recorded by others (2, 12). Also, tissue culture systems are somewhat simpler and are easier to handle than embryonated eggs.

As to pathogenesis, we are unable to define the sequential pathways by which RPV found its way to the final targets, namely skin, peripheral lymph nodes, spleen, liver, kidney, and lung. Nor do we know the cellular sites of earliest virus multiplication, or even other secondary targets necessary to the late and sometimes intensified viremia. The study of Bedson and Duckworth (2) suggests likely pathways; additional work is required for further orientations and precise definitions.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-06263 and Research Career Award K6-AI-13976 from the National Institute of Allergy and Infectious Diseases.

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