Acta virol. **30** : 75—80, 1985

ADSORPTION OF ECTYMA CONTAGIOSUM VIRUS TO CULTURED CALF TESTIS CELLS

M. TRAYKOVA, *R. ARGIROVA

Veterinary Institute of Immunology, Sofia
and *Institute of General and Comparative Pathology,
Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

Received October 10, 1984; revised May 14, 1985

Summary. — The adsorption of ectyma contagiosum virus (ECV) to calf testis cells was examined by attachment of ^{3}H-thymidine-labelled ECV to calf testis cell monolayers and endpoint titration. The adsorption rate for ECV was calculated to be 5×10^{-7} cm$^3\times$ $\times$ min$^{-1}\times$ cell^{-1}. ECV absorption was almost complete within 30 min though never exceeded more than 70% of the virus input.

Key words: ectyma contagiosum virus (ECV); adsorption; calf testis cells

ECV, a member of family Poxviridae, usually infects sheep, lambs and goats, causing contagious pustular dermatitis. Rarely, it is transmitted to humans who develop pustules on their fingers. Although ECV has been studied by many investigators (Sawhney, 1966; Nagington, 1968; Plowright *et al.*, 1969; Sawhney and Toschkov, 1971, 1972; Kluge *et al.*, 1972; Precausta and Stellman, 1973; Rossi, 1973; Traykova, 1982, 1983), the spread of ECV infection in vivo and in vitro remains open to discussion. It stimulated us to reexamine the cell-virus interactions with special emphasis on ECV adsorption to sensitive cells.

Calf testis cells were maintained in 199 medium supplemented with 10% calf serum and antibiotics. Subcultures were plated ($2 . 10^5$ cells/ml) and 24 hr later as monolayers infected with ECV in its 39th—46th cell culture passages. When maximum CPE developed, the tissue culture fluid was collected and stored at —30 °C. The virus was labelled by adding ^{3}H-thymidine (370 KBq; Prague, Czechoslovakia) 24—48 hr post-infection (p.i.), i.e. at the time of visible CPE. ECV was purified by centrifugation for 20 min at 1000 rev/min to remove the debris, followed by concentration of the supernatant for 2 hr at 50 000 rev/min in 3×35 mlj rotor (VAC-601-DDR). To obtain tenfold concentrated virus, the pellets were resuspended in Tris-HCl 0.01 mol/l, pH 7.3. After ultrasound treatment (30 sec), the concentrate was centrifuged through 36% sucrose (7 ml) for 40 min at 36 000 rev/min (3×35 ml rotor). The pellet was resuspended in the same buffer to give a final 100× virus concentration. An aliquote of ultrasouged treated material was layered on preformed 20—70 % sucrose gradient and spun for 2 hr at 50 000 rev/min. The gradient was separated into 20—22 fractions. Refraction index and 5% TCA inprecipitable radioactivity of these fractions were measured.

Measurement of virus adsorption was made either by attachment of ^{3}H-thymidine labelled ECV to or by endpoint titration method of the same virus in 2-day-old monolayers of calf testis cells. In adsorption experiments measuring uptake of ^{3}H-thymidine labelled virus, the cells were washed with Hanks solution and 20 microlitres of labelled virus (20 000—30 000 c.p.m.) were added to each culture (approx. 10^6 cells). After different periods of adsorption at 27 °C the

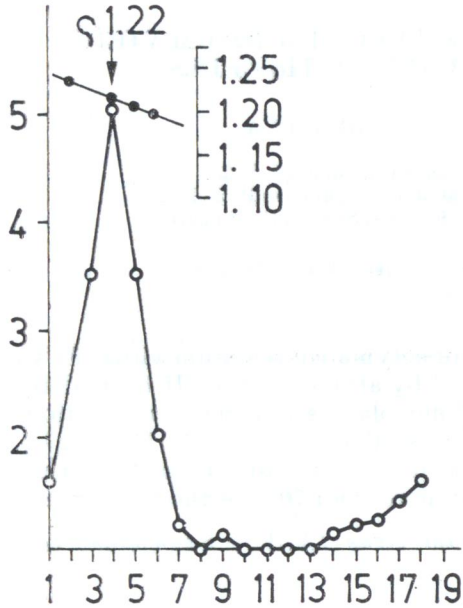

Fig. 1.
Analysis of ^{3}H-ECV in sucrose gradient (20—70%)
Abscissa: c.p.m. $\times 10^{-3}$; ordinate: fraction number.

unattached virus was removed and collected into 1 ml ice-cold Hanks solution, precipitated in 5% ice-cold TCA and filtered through millipore membrane filters (220 nm) for determination of radioactivity. For every experimental period 3 tube cultures were used from which the average value of c.p.m. was calculated.

In adsorption experiments using end-point titration method as a mode of quantitation, 4 tubes per dilution were infected with 0.2 ml virus for the same adsorption periods (multiplicity of infection about 1 ID_{50}/cell). The cells were observed for CPE at 24, 48, and 72 hr p.i. The virus titre was calculated according to Reed and Muench.

Analysis of ECV attachment was preceded by determination of buoyant density of ^{3}H-labelled ECV used for adsorption experiments (Fig. 1). Three samples of 3 labelled viruses were prepared for 3 adsorption experiments in 20—70% sucrose gradients. Every time only one peak of radioactivity was observed, the buoyant density being 1.22—1.23 g/ml. The titre of labelled purified virus in calf testis cells was $10^{6.5}$ ID_{50}/0.2 ml.

Table 1 shows the results of three adsorption experiments of ^{3}H-thymidine ECV to calf testis cells at 37 °C, representing the amount and proportion of unattached virus (in c.p.m. and %) at different adsorption intervals. Then the amount of adsorbed virus was the difference between the radioactivity of labelled virus (input c.p.m. = 100%) and unattached virus (in c.p.m. and %) at the given adsorption interval. As follows from Table 1, during 10 min 50—54% of labelled virus was already adsorbed to the cells, and its amount did not increase substantially at 20, 30 and 60 min. At 120 min about 65% of labelled virus has been adsorbed; almost the same value (68%) was measured by 180 min. The adsorption rate of sucrose-purified ^{3}H-thymidine labelled virus was never total, the maximum reached 68%.

Table 1. Adsorption of ^{3}H-ECV to calf testis cells at 37 °C

No. of experiment	Adsorption time (min)	Radioactivity of the input virus material (c.p.m.)	Unatached virus	
			c.p.m.	%
1	10	30 826	16 682	54
	20		14 633	47
	30		15 233	49
	60		13 368	43
	120		9 832	31
2	10	25 865	14 675	50
	20		12 563	48
	30		13 031	50
	60		11 843	45
	120		8 515	33
3	10	8 607	5 054	51
	20		3 593	40
	30		3 406	39
	60		4 577	53
	90		3 030	35
	120		3 468	40
	180		3 268	36

Based on these, Fig. 2 illustrates the attached virus (%) for different adsorption periods. The linearity of adsorption makes possible the calculation of adsorption rate (K_a) according to the formula of Levin and Sagik (1956):

$$K_a = \frac{2.3 \ln \frac{V_o}{V_t}}{nt},$$

where V_o — is the quantity of input virus (c.p.m.);
V_t — is the quantity of virus adsorbed (c.p.m.) for period t (difference between the input unattached virus within the t interval).
n — number of cells used in the adsorption experiment (in average 10^6 cells)
t — adsorption period in min

Using this formula we found that K_a for ECV to calf testis cells was approximately 5×10^{-7} cm^3 $\times$ min^{-1} $\times$ cell^{-1}.

The adsorption rate was studied in parallel using the endpoint titration method of labelled ECV in calf testis cells. After the given adsorption time the cells were observed for CPE at 24, 48 and 72 hr p.i. (Fig. 3). Whereas at 24 hr p.i. the virus titre was lower by 1.5 log unit per ml when ECV was

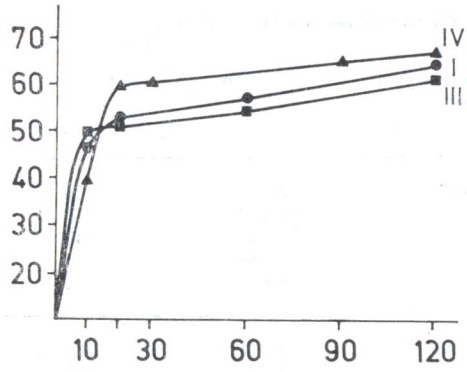

Fig. 2.
Adsorption kinetics of ^{3}H-ECV measured by attachment of labelled virus (%) to calf testis cells at 37 °C
Abscissa: adsorption time (min); ordinate: adsorbed ^{3}H-ECV (%).

allowed to adsorb for 10 min in comparison with 30, 60 and 120 min adsorbtion time, respectively. At 48 hr p.i. this difference decreased to 1 log/cm^3, and at 72 and 96 hr no difference was detectable, the virus titre reaching 5.5 $TCID_{50}$/cm^3 for all four adsorption times.

A number of studies dealing with adsorption of different viruses using different methods has been published. Determination of the amount of input virus as well as that of virus adsorbed within the t interval along with determination of the cell number at the moment of virus addition makes it possible to define the adsorption rate for every virus-cell system. Recently the wide application of radiolabelled virus makes it easier to monitor the adsorbed virus. The checking of virus particles (or their components) taking

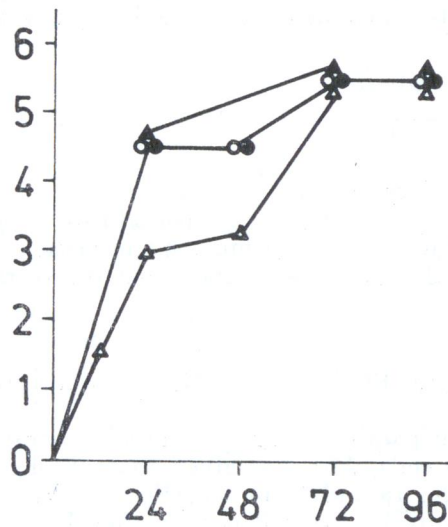

Fig. 3.
Reproduction dynamics of ECV in calf testis cells after different adsorption periods
△ 10 min; ▲ 30 min; ● 60 min; ○ 120 min.
Abscissa: hr p.i.; ordinate: ID_{50}/ml.

part in adsorption predicts any successful adsorption study. Fig. 1 demonstrates, that the radioactivity was bound mainly to particles of buoyant density of 1.22—1.23 g/ml. Gussev (1969) estimated in the bioassay that ECV adsorbed rapidly to lamb cells. He found that within 30—60 min about $90 \pm 5\%$ of the input virus had already become attached. In general, our data confirmed those of Gussev, but we have never found such a high proportion of virus adsorption. A possible explanation might be in different methods and/or cells used for adsorption.

Knowledge of adsorption rate for ECV enables to compare it with those of other viruses with known adsorption rates. So, ECV adsorbes more rapidly to calf testis cells than, for example, mouse oncornaviruses to different kinds of cells (K_a for mouse oncornaviruses is approx. 4.6×10^{-10} $cm^3 \times min^{-1} \times cell^{-1}$ (Argirova *et al.*, 1974), tick-borne encephalitis virus ($K_a = 3-6 \times 10^{-10}$ $cm^3 \times min^{-1} \times cell^{-1}$) (Altstein, 1963), Newcastle disease virus ($K_a = 2-5 \times 10^{-9}$ $cm^3 \times min^{-1} \times cell^{-1}$) (Levin and Sagik, 1956) or Mengo virus ($5-7 \times 10^{-8}$ $cm^3 \times min^{-1} \times cell^{-1}$) (Brownstein and Graham, 1961). For ECV, Mengo and vaccinia viruses the particle attachment was almost complete within 20 min and after a 120 min incubation it took 70%, 90% and 90%, respectively (Argirova *et al.*, 1974; Payne and Norrby, 1978). Under the same conditions 81% of influenza and 98% of poliomyelitis virus adsorbed to cell surface (Solovjov and Balandin, 1973).

There was no significant difference in ECV attached within 30 and 120 min intervals (as measured by bioassay). As follows from these data, 30 min are sufficient to achieve optimal ECV adsorption. Since the nature of ECV adsorption, especially the existence of specific attachment site—receptor interactions is not clear enough, further investigations using labelled ECV are desirable.

References

Altshtein, A. (1963): Study of acute and chronic infections induced by tick-borne encephalitis virus in tissue culture (in Russian). Doctor Thesis, Moscow, 1963.

Argirova, R., Zakharova, L., and Altshtein, A. (1974): Adsorption and reproduction of mouse sarcoma virus in cell systems differing in sensitivity to its transformation-destructive effect (in Russian). *Vop. Virus.* **19**, 333—338.

Brownstein, B., and Graham, A. (1961): Interaction of Mengo virus with L-cells. *Virology* **14**, 303—307.

Gussev, B. (1969): Reproduction of *Ecthyma contagiosum* virus — K-I strain in tissue culture (in Russian), Thesis, Frunze.

Kluge, J., Cheville, N., and Peery, T. (1972): Ultrastructural studies of CE in sheep. *Am. J. vet. Res.* **33**, 1191—1200.

Levine, S., and Sagik, B. (1956): The interactions of Newcastle disease virus (NDV) with chick embryo tissue culture cells. Attachment and growth. *Virology* **2**, 57—68.

Nagington, J. (1968): The growth of Paravaccinia viruses in tissue culture, *Vet. Rec.* **82**, 477—478.

Payne, L., and Norrby, E. (1976): Adsorption and penetration of enveloped and naked vaccinia virus particles. *J. Virol.* **27**, 19—27.

Plowright, W., Witcomb, M., and Ferris, R. (1969): Studies with a strain of contagious pustular dermatitis virus in tissue culture. *Arch. ges. Virusforsch.* **9**, 214—231.

Precausta, P., and Stellman, C. (1973): Isolation and comparative study in vitro of 5 strains of contagious ecthyma of sheep. *Zentbl. Vet.-Med.* B **20**, 340—355.

Reed, L., and Muench, H. (1938): A simple method of estimating 50 per cent end-point. *Am. J. Hyg.* **27**, 493–497.

Rossi, G. (1973): Adaptation of the virus of CE to cellular substrates of avian origin. *Vet. Ital.* **24**, 218–222.

Sawhney, A. (1966): Studies on the virus of CPD in sheep. Doctor Thesis, Publishing House of Bulgarian Academy of Sciences, Sofia.

Sawhney, A., and Toschkov, A. (1971): Propagation of CPD virus in HeLa cell cultures. *Indian J. exp. Biol.* **9**, 512–514.

Sawhney, A., and Toschkov, A. (1972): Action of organic lipid solvents saponin and trypsin on the virus of *Ect. contagiosum*. *Indian J. exp. Biol.* **10**, 234–235.

Solovjov, V., and Balandin, I. (1973): *Cell and Virus* (in Russian). Meditsina, Moscow, 190 pp.

Traykova, M. (1982): Experimental adaptation of *Ecthyma contagiosum virus* in tissue cultures (in Bulgarian). *Vet. Med. Nauki* **10**, 18–25.

Traykova, M. (1983): Effect of different physicochemical factors on *Ecthyma contagiosum* virus (in Bulgarian). *Scientific works of NJPVMII* **1**, 359–366.