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MULTIPLICATION OF *COXIELLA BURNETI* IN VIRUS-INFECTED AND INTERFERON-TREATED CELL CULTURES

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Summary. — In chick embryo cell (CEC) cultures infected with tick-borne encephalitis (TE) virus, *Coxiella burneti* multiplied to lower titres and the proportion of *C. burneti*-infected cells was lower than in control CEC cultures. Treatment of CEC or L cell cultures with homologous virus-induced chick or mouse interferon had no effect on the multiplication of *C. burneti* in these cell cultures.

The present knowledge about the effect of viral infection on subsequent rickettsial challenge has been limited to the observation of resistance against a lethal dose of *C. burneti* in guinea pigs previously infected with influenza virus (Jansen *et al.*, 1965). Nothing is known about the effect of interferon on the multiplication of rickettsiae. We have investigated, therefore, whether previous infection of cell cultures with a weakly cytopathic virus or their treatment with interferon induced in species-specific cell substrates affects the multiplication of *C. burneti* in these cultures, in particular the titre of *C. burneti* and the degree of its multiplication at given time intervals after inoculation.

Two-day CEC cultures grown in tubes with inserted coverslips were inoculated with 2 dilutions (input multiplicity of infection — MI — of 200 and 2 LD_{50} per cell) of mouse brain suspension of the weakly cytopathic Hy-M line (Libíková and Stanček, 1965) of the Hypr strain of TE virus. After 90 minutes' adsorption at 37° C the inoculum was removed and the cultures washed twice with phosphate buffered saline (PBS) and supplied with maintenance medium. After 3 days of incubation at 37° C and removal of medium, the TE virus-infected and control uninfected CEC cultures of the same age were used for titration of *C. burneti*, strain Nine Mile. The CEC cultures were infected with 1 ml of serial tenfold dilutions of a 10% *C. burneti*-infected chick embryo yolk sac suspension, using 4 tubes for each dilution. After 2 hours' adsorption at 37° C, the rickettsial inoculum was removed and the cultures were washed thrice with PBS and supplied with fresh maintenance medium. On the 5th day of incubation at 37° C, the coverslips were removed, air-dried, and stained according to Giemsa. After microscopic examination for the presence of *C. burneti*, the titre of the latter ($TCID_{50}$/ml) was calculated by the formula of Reed and Muench. TE virus multiplication at the time of challenge of the CEC cultures with *C. burneti* was checked by assaying the culture fluids taken 3 days after virus inoculation in interference tests in fresh CEC cultures against 500 $TCID_{50}$ of Western equine encephalomyelitis (WEE) virus (Vilček, 1964).

In a further experiment, 1 and 5 days old CEC cultures were infected with the Hy-M line of TE virus (MI = 20 LD_{50} per cell). After 3 days at 37° C, these and uninfected CEC cultures of the same age were inoculated with the Nine Mile strain of *C. burneti* (MI = 20 EID_{50}, determined by titration in chick embryo yolk sacs). The degree of *C. burneti* multiplication was estimated in two tubes each at 24-hour intervals until the 4th day after challenge with *C. burneti*. After staining according to Giemsa, the proportion of *C. burneti*-containing cells was calculated;

200 cells were examined in each CEC culture. Interferon in media taken from TE virus-infected and control CEC on the day of challenge with *C. burneti* and heated for 1 hour at 60° C to inactivate TE virus was titrated in fresh CEC cultures against 200 $TCID_{50}$ of WEE virus.

The effect of interferon on *C. burneti* multiplication (on the titre and degree of multiplication) was investigated in parallel in CEC and L cell cultures. Before inoculation with *C. burneti*, both cultures were incubated for 24 hours at 37° C with 64 units of species-specific interferon. Medium taken from CEC cultures 3 days after inoculation with TE virus, heated for 1 hour at 60° C to inactivate the virus, served as source of chick interferon. Washings from peritoneal cavities of mice, 6 hours after intraperitoneal inoculation of $10^{7.2}$ plaque forming units of Newcastle disease virus (Lackovič and Borecký, 1965), in which the virus was inactivated by dialysis for 72 hours against citrate buffer pH 2, served as source of mouse interferon. The latter was titrated in L cell cultures against 200 $TCID_{50}$ of mouse encephalomyocarditis virus. Titration and estimation of the degree of multiplication of *C. burneti* in interferon-treated and parallel control cell cultures was carried out as described above.

In all experiments, culture media without antibiotics were used. The growth medium was medium 199 supplemented with 10% heated calf serum; medium 199 with 2% heated calf serum was employed as maintenance medium.

Table 1. Titration of C. burneti in TE virus-infected CEC cultures

TE virus MI (LD_{50}/cell)	TE virus titre* (log ifD_{50}/ml)	*C. burneti* titre (log $TCID_{50}$/ml)
200	8.5	3.0
2	7.6	3.3
0 (control)	—	4.8

* Titre of TE virus in culture fluids taken 3 days after inoculation at the time of challenge with *C. burneti*.

The results (Table 1) showed that, although *C. burneti* multiplied also in CEC cultures previously infected with TE virus, the multiplication of the latter interferred with that of *C. burneti*. This interference was manifested by a decrease of 1.3 or 1.8 log units in the titre of *C. burneti* as compared with its titre in control CEC cultures. It is probable that the degree of this interference depends on the dose of TE virus inoculum, because the decrease

Table 2. Course of C. burneti multiplication in Te virus-infected CEC cultures

CEC cultures		Per cent of cells infected with *C. burneti* on days after challenge				Interferon titre*
Age	TE virus	1	2	3	4	
1 day	Infected	1	4	11	26	8
	Uninfected controls	7	28	57	71	0
5 days	Infected	< 1	1	5	17	32
	Uninfected controls	5	23	49	68	0

* Units of interferon per ml medium harvested from the cultures 3 days after inoculation with TE virus, i.e. on the day of challenge with *C. burneti*.

in *C. burneti* titre was greater in CEC cultures inoculated with the greater dose of TE virus (MI = 200).

A further manifestation of the interference between TE virus and *C. burneti* was a decrease in the proportion of *C. burneti*-infected cells in CEC cultures previously infected with TE virus. This decrease was somewhat more marked in older CEC cultures, in which TE virus had induced more interferon than in younger CEC cultures (Table 2).

In spite of the fact that there was some interference between TE virus and *C. burneti* in CEC cultures, the addition of 64 units of species-specific TE virus-induced chick interferon to CEC cultures before their inoculation with *C. burneti* did not affect either the titre of *C. burneti* or the proportion of *C. burneti*-infected cells. Similarly, treatment of L cell cultures with 64 units of mouse interferon had no effect on *C. burneti* multiplication in these cultures.

Since the doses of exogenous interferon used did not prevent the multiplication of *C. burneti*, it is possible to assume that the interference between TE virus and *C. burneti* in CEC cultures probably is mediated by a mechanism other than that of interferon. It is not excluded, however, that in TE virus-infected cells even lower amounts of endogenous interferon induced by this virus might inhibit *C. burneti* multiplication. It is also possible that doses of exogenous interferon higher than those used in the present experiments might be effective. This assumption is supported by the finding that amounts of interferon greater than those inhibiting virus multiplication are needed to reduce the multiplication of Chlamydiae, which stand close to Rickettsiae (Hanna *et al.*, 1966; Mordhorst *et al.*, 1966).

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