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Short communication

The difference of energy metabolism between persistent and cytocidal virus infections

Liu Yuwen [a], Zhang Heng [a], Wang Cunxin [a], Li Jie [a], Li Huitin [a], Li Zhaoyang [b], Wu Jibin [b], Zheng Congyi [b,*]

[a] *College of Chemistry and Molecule Science, Wuhan University, Wuhan 430072, PR China*
[b] *College of Life Science, Wuhan University, Wuhan 430072, PR China*

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Abstract

In this study, microcalorimetric measurements were carried out on PK-15 cell line persistently infected by cholera swine fever virus (CSFV) and BHK-21 cell line infected by foot and mouth disease virus (FMDV). The aim of this investigation was to investigate the difference of energy metabolism between the different kinds of virus infections—persistent infection and cytocidal infection. The thermogenesis curves determined by using a LKB 2277 Bioactivity Monitor were significantly different. From these thermogenic curves, the maximum heat production rate P_m and total heat output Q are obtained. The results show that energy metabolism is different between persistent infection and cytocidal infection.

Keywords: Virus; Microcalorimetry; Metabolism; Persistent infection; Cytocidal infection

1. Introduction

When viruses infect cells, two general outcomes are possible. Either the infected cells are killed, but produce a large yield of virus (a lytic interaction) or the cell continues to multiply while making small amounts of virus [1]. Virus infections thus can be classified into cytocidal infection (lytic interaction) or persistent infection based on their effects on the host cells. The most easily recognized is a destructive or cytocidal effect characterized by extensive damage to many different cell organelles. In persistent infection, cells continue to function for some time after infection and at least in one case to grow and divide while producing virus. Because of the ease with which the virion-specific material can be followed and the slower development of knowledge and methods that allow a detailed comparison of cellular processes in normal and infected cells, investigations of the events following virus infection of animal cells have dealt more with the synthesis of new virion components [2] than with effects on host cells.

In this study, a PK-15 cell line persistently infected by a field-isolated strain of classical swine fever virus (CSFV39) (CSFV39-PK-15 cell line) [3] and a BHK-21 cell line with cytocidal virus infection by foot and mouth disease virus (FMDV) [4] were investigated by microcalorimetry. The metabolic processes of normal PK-15 and BHK-21 cells were also investigated. The shapes of the thermogenesis curves of persistent infection and cytocidal infection processes are significantly different. The maximal thermal power (P_{max}), the mean thermal power (P_{mean}) and total heat

* Corresponding author. Fax: +86-27-87647617.
E-mail address: ipc@whu.edu.cn (Z. Congyi).

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production during the measurement period (Q), all are indicators that the multiplication mechanisms of virus in these two kinds of infections are significantly different.

2. Materials and methods

2.1. Instrument

An LKB-2277 Bioactivity Monitor (Thermometric AB, Sweden) was used in this experiment. The detection limit is 0.15 μW and the baseline stability (over a period of 24 h) is 0.2 μW. The performance of this instrument and the details of its construction have been previously described [5].

2.2. Materials

PK-15 cells, PK-15 cells persistently infected with CSFV39 (CSFV39-PK-15 cell line), BHK-21 cells, and FMDV were provided by China Center for Type Culture Collection, Wuhan University. Cultures in exponential growth phase are obtained by seeding 10^5 cell ml^{-1} PK-15 cells into T-25 plastic flask containing 8 ml medium at 37 °C, 5% CO_2 for 2 days. The medium consists of 90% DMEM medium (GIBCO Co., USA), 10% heat-inactivated fetal calf serum (GIBCO Co., USA) with 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin added (pH = 7.2–7.3). The same medium was used for the calorimetric experiments. Cells were counted by using a Thoma's hemocytometer.

2.3. Procedure

The thermogenesis curves of PK-15 and BHK-21 cell lines both uninfected and infected were recorded with the ampoule method with 3 ml glass ampoules. The reference ampoule contained 1 ml of culture medium, and the other contained 1 ml of sample. Each ampoule also contained 2 ml of air. The initial cell number was 10^5 cell ml^{-1} in all experiments. The measurement was carried out at 37 °C. All experiments have been repeated three times. All the procedures are the same except for an extra procedure of FMDV infection. BHK-21 cells cultured in the ampoule for 24 h were infected with 0.05 ml of virus. Following 30 min at 37 °C, the cells were washed twice with fresh DMEM and the 1 ml original medium

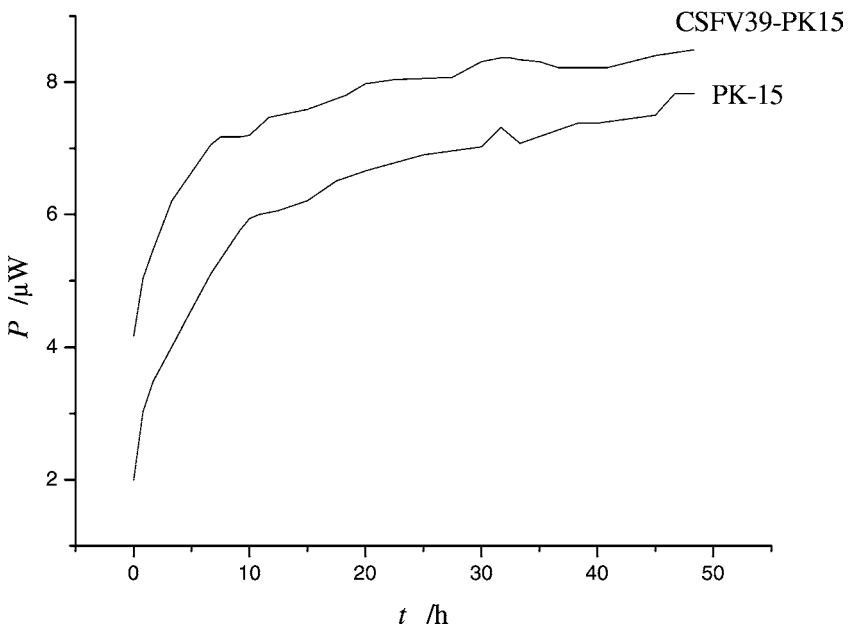

Fig. 1. Thermogenesis curves of PK-15 cells and CSFV39-PK-15 cells (persistent infection).

was put back into the ampoule. The ampoule was then sealed and put into the microcalorimeter with a reference ampoule to determine the thermogenesis curves.

3. Results

The thermogenesis curves of PK-15 cells and PK-15 cells persistently infected by CSFV39 (CSFV39-PK-15 cells) at 37 °C are shown in Fig. 1. Fig. 1 shows that the thermal power of CSFV39-PK-15 cells (persistent infection) is higher than that of PK-15 cells.

The total metabolic heat released during the experiment is given in Table 1. The maximum thermal power and the mean thermal power are also given in Table 1.

The thermogenesis curves of BHK-21 cells and BHK-21 cells infected by FMDV (cytocidal infection) at 37 °C are shown in Fig. 2. The shape of the thermogenesis curves of BHK-21 cells infected by FMDV is significantly different from that of normal BHK-21 cells. The exponential increasing phase and exponential declining phase clearly seen in the thermogenesis curves of BHK-21 cells infected by FMDV are not seen with normal BHK-21 cells. The exponential increase of thermal power with time cannot be explained by the growth of cells, it can only be caused by the action of FMDV on BHK-21 cells. The total heat released during the experiment, the maximum thermal power, and the mean power are given in Table 1.

Table 1
Analysis of themogenesis curves of PK-15 and BHK-21 cell lines both uninfected and infected

	Q (J)	P_{mean} (μW)	P_{max} (μW)
PK-15 cells	1.12 ± 0.05	6.47 ± 0.29	7.83 ± 0.39
CSFV39-PK-15 cells	1.34 ± 0.07	7.71 ± 0.40	8.49 ± 0.42
BHK-21 cells	4.10 ± 0.24	10.10 ± 0.60	13.65 ± 0.71
BHK-21 cells infected by FMDV	9.16 ± 0.41	27.5 ± 1.22	69.00 ± 3.58

4. Discussion

From Fig. 1, the shapes of the thermogenesis curves of normal PK-15 cells and CSFV39-PK-15 cells (persistent infection) are very similar, except that the curve of CSFV39-PK-15 cells is a little higher than

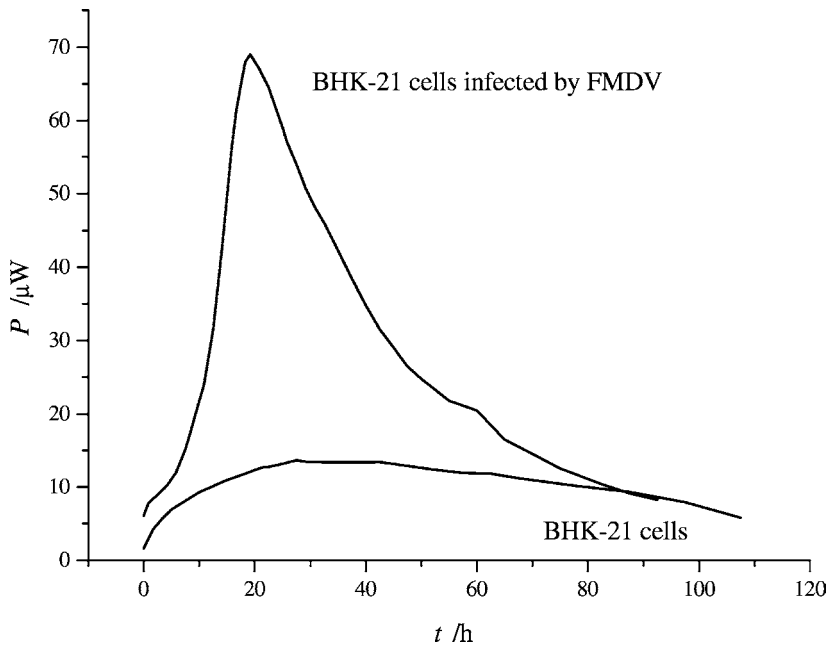

Fig. 2. Thermogenesis curves of BHK-21 cells and BHK-21 cells infected by FMDV (cytocidal infection).

that of the normal PK-15 cells. The total metabolic heat, the mean thermal power and the maximum thermal power of CSFV39-PK-15 cells are all about 20% larger than those of normal PK-15 cells. This indicates that virus which cause persistent infection accelerate many biochemical processes, but the action of the virus on the host cells is not severe on the whole. In Fig. 2, the shape of the metabolic thermogenesis curve of BHK-21 cells infected by FMDV is significantly different from that of normal BHK-21 cells. The total metabolic heat and the mean thermal power are three times that of the normal BHK-21 cells, and the maximum thermal power is fives times of that of the normal BHK-21 cells, showing the severity of cytocidal infections on the host cells. Though the changes in biochemical reactions in the host cells are very complicated after being infected by virus, microcalorimetry can be used to monitor the overall processes. The thermogenesis curves not only give information about metabolic heat, but also information about the thermokinetics of virus replication.

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References

[1] S.E. Luria, J.E. Darnell, D. Baltimore, A. Campbell, General Virology, third ed., Wiley, New York, 1978.
[2] M.L.P. Murphy, M.A. Forsyth, G.J. Belsham, J.S. Salt, Localization of foot-and-mouth disease virus RNA by in situ hybridization within bovine tissues, Virus Res. 62 (1999) 67–76.
[3] S.C. Ying, C.Y. Zheng, S.P. Qu, C.Y. Zhang, Z.S. Wang, Model establishment of the cell persistently infected by a field-isolated virulent strain of CSFV, Virologica Sinica 17 (2002) 77–81.
[4] H.L. Bachrach, Foot-and-mouth disease, Ann. Rev. Microbiol. 22 (1968) 201–244.
[5] J. Suurkuusk, I. Wadsö, A multichannel microcalorimetry system, Chem. Scr. 20 (1992) 155–163.