SHORT COMMUNICATION

Invasion by *Toxoplasma gondii* of ATP-depleted and ATP-restored Chick Embryo Erythrocytes

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(Received 7 June 1982; revised 26 July 1982)

Chick embryo erythrocytes (CEE) were treated with NaF and KCN to deplete their ATP levels, and their susceptibility toward *Toxoplasma gondii* invasion was examined. A marked reduction in the invasion rate was observed in ATP-depleted CEE but the rate was recovered in ATP-restored CEE. These results indicate that *T. gondii* invasion is dependent on the ATP level of the CEE. Consequently, energy-dependent host cell activity is considered to be prerequisite for the invasion of *T. gondii*.

INTRODUCTION

An obligatory intracellular protozoan parasite, *Toxoplasma gondii*, infects both phagocytic and non-phagocytic cells. Morphological observations have shown that the host cell intimately interacts with an invading *T. gondii* (Aikawa *et al.*, 1977). Jones *et al.* (1972) have proposed that *T. gondii* induces phagocytosis in non-phagocytic cells and the host cell engulfs the parasite. Since cell activity such as phagocytosis is energy dependent, it may be expected that *T. gondii* does not invade non-phagocytic cells whose ATP is depleted.

In our experiments, we used chick embryo erythrocytes (CEE) as host cells (Tanabe *et al.*, 1980). They were non-phagocytotic and could be manipulated by metabolic inhibitors so as to deplete their ATP (Frish *et al.*, 1973). *Toxoplasma gondii* easily invades CEE under simple experimental conditions in which cell culture medium and serum are excluded (Tanabe *et al.*, 1980). Accordingly, we examined the invasion of ATP-depleted CEE by *T. gondii* and found the invasion to be dependent on the host cell ATP level.

METHODS

Chick embryo erythrocytes (CEE) and the RH strain of *Toxoplasma gondii* tachyzoites were used. The preparation of CEE and parasites are described in our earlier papers (Tanabe *et al.*, 1978, 1980). Packed CEE (0.1 ml) were incubated for 8 h at 4 °C or 37 °C in 10 ml ATP-depletion medium [135 mM-KCl, 5 mM-NaCl and 10 mM-Na₂HPO₄. KH₂PO₄ at pH 7.4 (rPBS) with 10 mM-NaF and 1 mM-KCN]. Some samples were removed after 4 h incubation, washed with rPBS three times at 4 °C and further incubated for 4 h at 37 °C in ATP-restoration medium in which rPBS was supplemented with 1 mM-MgCl₂, 5 mM-glucose, 1 mM-adenine and 5 mM-inosine. As a control, CEE were incubated only in rPBS at 4 °C or 37 °C. No haemolysis occurred either during or after incubation under these conditions. The amount of cellular ATP was monitored with an ATP-test (Boehringer Mannheim).

Assay for *T. gondii* invasion of CEE was conducted according to our methods (Tanabe *et al.*, 1980) with a minor modification. Briefly, 2.5×10^6 CEE were incubated with 5×10^6 parasites per ml of a mixture containing 135 mM-NaCl, 5 mM-KCl, 1 mM-MgCl₂, 5 mM-glucose and 10 mM-HEPES at pH 7.8. After incubation for 3 h at 37 °C, the CEE were collected and the invasion rates determined by counting parasitized CEE on Giemsa-stained cell smears.

The statistical difference was examined by Student's *t*-test.

Table 1. *Invasion by Toxoplasma gondii of ATP-depleted and ATP-restored chick embryo erythrocytes*

Details of the assay conditions and procedures are given in the Methods. The results are the mean values for four experiments $\pm$ S.D.

Preincubation conditions	Invasion (%)	ATP content [$\mu\text{mol (ml cells)}^{-1}$]
rPBS for 8 h at 4 °C	12.2 $\pm$ 1.4	1.29 $\pm$ 0.23
rPBS for 8 h at 37 °C	10.3 $\pm$ 1.6	1.11 $\pm$ 0.19
ATP-depletion medium for 8 h at 4 °C	12.7 $\pm$ 1.2	1.07 $\pm$ 0.27
ATP-depletion medium for 4 h at 37 °C and then rPBS for 4 h at 37 °C	3.2 $\pm$ 1.2	0.25 $\pm$ 0.01
ATP-depletion medium for 8 h at 37 °C	0.7 $\pm$ 0.3	0.11 $\pm$ 0.01
ATP-depletion medium for 4 h at 37 °C and then ATP-restoration medium for 4 h at 37 °C	8.5 $\pm$ 1.1	1.94 $\pm$ 0.12

RESULTS

Toxoplasma gondii invasion rates and ATP content of CEE treated with metabolic inhibitors are shown in Table 1. No significant difference in invasion rate was observed among the control groups, i.e. CEE incubated in rPBS at 4 °C or 37 °C, or those in ATP-depletion medium at 4 °C. In contrast to the controls, a marked reduction in the rate was observed in ATP-depleted CEE incubated at 37 °C. The reduction was 69–75% of the control in CEE treated for 4 h ($P < 0.001$) and 93–94% in CEE treated for 8 h ($P < 0.001$). Parasites co-incubated with ATP-depleted CEE were motile when observed with a phase-contrast microscope attached to a thermobox at 37 °C. This shows that ATP-depleted CEE do not contain residual inhibitors which would inhibit parasite activity. The ATP content of these CEE also considerably decreased ($P < 0.01$). On the other hand, a recovery of the invasion rate to that of the control level was seen in ATP-restored CEE. A statistically insignificant difference in the rates between the restored CEE and control CEE was detected ($P > 0.05$). The ATP content in restored CEE was regained beyond that of the control level ($P < 0.05$). The reason for this increase is not known.

DISCUSSION

There has been some dispute as to the manner in which *T. gondii* enters the host cells. In the anterior part of the body, *T. gondii* has special organelles such as rhoptries and micronemes, which are thought to assist the entry of parasites into a host cell (Lycke *et al.*, 1975). Bommer *et al.* (1969) considered that active penetration is the mode by which parasites enter a host cell. This view is supported by the fact that treatment of *T. gondii* with KCN inhibits their entry into host cells (Werk & Bommer, 1980). On the other hand, Jones *et al.* (1972) proposed that phagocytosis induced by a parasite is a primary mechanism for entry; this is supported by experiments of Ryning & Remington (1978) in which pretreatment of bladder tumour cells with an inhibitor of microfilament activity, cytochalasin D, prevents parasite entry. However, their studies leave the possibility that cytochalasin D acts not only on the host cells but on parasites. Phagocytosis is a complex cellular activity involving rearrangement of cellular contractile protein, microfilaments, and is supported by ATP. Metabolic inhibitors abolish the phagocytotic uptake of particles (Cohn, 1970).

We found that *T. gondii* invasion decreases in ATP-depleted CEE but is recovered in ATP-restored CEE. CEE are non-phagocytotic under normal circumstances. We are not certain whether *T. gondii* induces CEE to undergo phagocytosis. However, it seems less likely that phagocytosis can take place in ATP-depleted CEE. Host cell membrane movement at the site of *T. gondii* attachment prior to invasion would not be simply a passive event but an active process requiring cellular ATP. Thus, it is our opinion that a host cell response, such as phagocytosis, is necessary to allow for parasite invasion.

Whatever the mechanism of the entry is, our present study indicates that *T. gondii* invasion of CEE is dependent on the host cell ATP level. It is of interest to see if this is true for other non-phagocytic cells or not.

The authors are grateful to Professor S. Takada for his encouragement of this work and to Dr Y. Funae for providing use of his facilities.

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