SHORT COMMUNICATION

Attempts to Infect Plant Protoplasts with *Toxoplasma gondii*

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The interaction of tachyzoites of *Toxoplasma gondii* with plant protoplasts was compared with their interaction with cultures of HeLa cells. Unlike the HeLa cells, the plant protoplasts were refractory to invasion under all conditions tested. The lack of invasion was not an artefact of the protoplast isolation technique. This is the first description of a cell type refractory to *Toxoplasma* invasion; hence possible mechanisms for the specificity of parasite–host cell interaction can now be studied.

INTRODUCTION

Toxoplasma gondii infects an unusually broad range of host cells: it will infect cells of vertebrates (Doran, 1973), including those of fish (S. Takada, personal communication), as well as insect cells (Buckley, 1973). So far, no cell type totally resistant to *Toxoplasma gondii* infection has been reported. The discovery of a cell line resistant to infection might introduce new aspects on *Toxoplasma gondii* invasion. An experimental model using plant protoplasts as target cells for *Toxoplasma* parasites is described in this paper. Plant protoplasts were chosen since they differ from animal cells in fine structure and composition of the membrane, including the cell surface (Evans, 1976).

METHODS

Organisms. *Toxoplasma* parasites were isolated from the peritoneal cavity of mice as described previously (Werk & Bommer, 1978). *Candida albicans* cultures were obtained from the mould culture collection of the Institut für Hygiene und Medizinische Mikrobiologie der Universität Würzburg (Professor Seeliger).

Cell and protoplast cultures. Cover-slip cultures of HeLa cells were prepared according to Werk & Bommer (1980).

Plant protoplasts were prepared as follows. Young leaves of *Catharanthus roseus* (Apocynaceae) and *Brassica napus napus* (Brassicaceae) were sterilized for 10 s in 70% (v/v) ethanol and then for 30 min in 5% (v/v) sodium hypochlorite solution. After washing three times with deionized water, the leaves were cut into small pieces and left in 0.55 M-mannitol for 30 min at room temperature to let cell debris settle. The leaf material was incubated in Erlenmeyer flasks for about 14 h at 22 °C with a cell wall digestion mixture containing 0.4% (w/v) Cellulase 'Onozuka' R10, 0.1% Mazerozyme R10 (both enzymes from Kinki Yakult, Nishinomiya, Japan) and 79.3 µM-Ca(H₂PO₄)₂, in 0.55 M-mannitol adjusted to pH 7.0 with KOH. Protoplasts were then obtained by shaking the flasks for 30 min. The protoplast suspension was passed sequentially through two nylon meshes (mesh size 125 and 40 µm) and collected by centrifugation (200 g, 10 min). The procedure was repeated three or four times and the enzyme solution was replaced by 0.55 M-mannitol or a modified protoplast culture medium (Nitsch & Nitsch, 1969) containing 9.1% (w/v) mannitol, 10% (w/v) glucose and 20% (w/v) sucrose.

Infection studies. The experimental schedule, which also indicates the ratio of host 'target' cells to parasite cells, is described in Table 1. Host cells were presented to *Toxoplasma* either in 0.55 M-mannitol or in plant protoplast culture medium. The infection period for plant protoplasts was 8 h at either 37 °C or 26 °C. Controls included an

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Table 1. *Infection experiments with Toxoplasma gondii and plant cell protoplasts or HeLa cells*

Plant cell protoplasts were exposed to *T. gondii* (or *C. albicans*) for 8 h in 0.55 M-mannitol or in a modified protoplast culture medium; HeLa cells were exposed for 30 min in mannitol (see Methods).

'Target' cell (no.)	'Effector' cell (no.)	Infection of target cells (% $\pm$ S.E.) in:			
		Mannitol		Culture medium	
		26 °C	37 °C	26 °C	37 °C
<i>Catharanthus roseus</i> (10^5)	<i>Toxoplasma gondii</i> (10^7)	0	0	0	0
<i>Brassica napus napus</i> (10^5)	<i>Toxoplasma gondii</i> (10^7)	0	0	0	0
<i>Catharanthus roseus</i> (10^5)	<i>Candida albicans</i> (10^5)	0	0	0	0
HeLa cell culture, untreated	<i>Toxoplasma gondii</i> (5×10^6)	NT	27.9 ± 7.0	NT	NT
HeLa cell culture, enzyme treated	<i>Toxoplasma gondii</i> (5×10^6)	NT	34.0 ± 7.5	NT	NT

NT, Not tested.

assay of protoplast integrity before and after exposure to *Toxoplasma*, as examined by phase-contrast microscopy. *Toxoplasma* viability in the protoplast medium after 8 h was checked by Trypan blue dye exclusion (Paul, 1975).

HeLa cells were treated for 30 min with the enzyme mixture for protoplast preparation before exposure to *Toxoplasma* parasites. The infection rate over 30 min for treated and untreated HeLa cells was assayed as described before (Werk & Bommer, 1980).

RESULTS

Enzymically prepared mesophyll protoplasts of *Catharanthus roseus* and of *Brassica napus napus* were infected under the experimental conditions described in Table 1. The process was followed by phase-contrast microscopy for 8 h. Plant protoplasts were incubated with cells of *Candida albicans*, which are comparable in size with those of *Toxoplasma*, in order to determine whether or not phagocytosis occurred. Under the experimental conditions chosen, no intracellular yeast cells were observed, indicating that the plant protoplasts were non-phagocytotic.

Fresh preparations of plant protoplasts were incubated with freshly isolated *Toxoplasma*. During the first 4 h a steady decrease of mobility and active contact of the parasite was observed. After that time no mobility and contact were observed. Under no experimental conditions tested were any protoplasts found to be infected (Table 1). In order to exclude the possibility that the plant cell wall digestion mixture had an inhibitory effect, HeLa cover-slip cultures pretreated with the same enzyme mixture were overlaid with *Toxoplasma*. The infection rate of the treated HeLa cells was 27.9% as determined by phase-contrast microscopy. This was not significantly different from the infection rate of untreated controls (34.0%) (Table 1).

DISCUSSION

No animal cell type, probably including erythrocytes (Schupp *et al.*, 1978), has been found to be resistant to *Toxoplasma* invasion. However, different degrees of susceptibility of host cells to infection have been reported, due either to the type of target cell (Tanabe *et al.*, 1979) or to modification of the membrane microviscosity by chemical factors, e.g. lidocaine (Werk & Bommer, 1980).

Plant protoplasts may be of special interest as potential target cells, since their membrane structure and composition, in terms of cholesterol/phospholipid ratio, are generally accepted

to be different from those of mammalian cells (Evans, 1976). Tanabe *et al.* (1979) considered the cholesterol/phospholipid ratio to be one of the reasons for the variable susceptibility to infection of some host cells like maturing red blood cells. After enzymic digestion of the polysaccharide cell wall, the plant cell, now converted into a protoplast, is surrounded by a unit membrane (Evans, 1976). However, the fragility of this membrane suggests that its viscosity is not very high (unpublished observations). Thus, the plant protoplasts should be readily infected. However, in this study plant protoplasts were found to be resistant to both adhesion of and infection by *Toxoplasma*. Electron microscopic examinations have also failed to show intracellular parasites (R. Werk, R. Dunker & S. Fischer, unpublished observations). This lack of infection was not due to inhibition by small amounts of enzymes remaining after digestion of the plant cell wall, since the infection rate of HeLa cells treated with the same enzymic cell wall digestion mixture was almost the same as that of untreated HeLa cells. Incubation with *Candida albicans* showed that under the experimental conditions studied, phagocytosis was absent in our plant protoplasts.

It can thus be concluded that plant protoplasts are the only cell type so far found to be totally resistant to *Toxoplasma gondii* infection; invasion of mature erythrocytes as reported by Schupp *et al.* (1978) is still a matter of controversy (Tanabe *et al.*, 1979). Further studies are necessary to analyse the mechanism of resistance of plant protoplasts to infection with *Toxoplasma gondii*, and these may throw light on the factors required for infection of susceptible cells.

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