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Quantitative Comparison of Infection of Neural Cell and Fibroblast Monolayers by Two Strains of *Toxoplasma gondii* (42587)

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Abstract. Disease caused by the coccidian *Toxoplasma gondii* can be confined to the central nervous system, although the parasite is capable of infecting all organ systems. To determine whether neural cells are differentially susceptible to infection and destruction by *T. gondii*, infection of neonatal mouse brain monolayers was compared to infection of human fibroblast monolayers under the same conditions with equal inocula of two parasite strains. In preliminary experiments there was no difference in total parasite yield or in plaques per monolayer between rodent and human cells. A standardized inoculum of *T. gondii* RH strain caused 35.6 ± 6.4 (SD) plaques per well in neural explant monolayers compared to 39.3 ± 12.5 plaques per well in fibroblasts. *T. gondii* P strain produced 35.6 ± 8.9 infected foci per well in neural cells compared to 32.6 ± 9.3 foci in fibroblasts. Intrinsic properties of neural cells do not appear to cause a higher rate of infection than that in nonneural cells.

The intracellular parasite *Toxoplasma gondii* causes a spectrum of disease including lesions of the central nervous system. Although the parasite can infect cells of any organ system, the central nervous system is often the site of the most serious involvement (1, 2), and patients have been reported with toxoplasmosis confined to the brain (3–10). *T. gondii* enters cells by an active process (reviewed in (11)) and apparently can infect and replicate within any nucleated vertebrate cell. The reason for the differential severity of pathological involvement between organ systems is not well understood, but one possibility is that neural cells are more receptive to attack by this parasite than other cell types. An *in vivo* model of cerebral infection has recently been reported (12). In such a model the influences of local immunity and the factors which control cyst formation could confound the determination of differential cell susceptibility. Therefore, in order to test whether neural cells are more susceptible to infection than are nonneural cells *in vitro*, I compared infection of monolayers derived from explants of neonatal mouse brain to infection of monolayers of the fibroblasts in which the *T. gondii* had been maintained. Two strains of *T. gondii* with different growth characteristics were used in this assay to control for virulence properties associated with individual strains. No difference was found between cell types or between parasite strains which could explain the propensity of the central nervous system to pathological involvement in toxoplasmosis.

Materials and Methods. Neural explants of the brains of 12-hr-old 129/J mice were placed in 12-well plastic culture plates (Costar) in Waymouth's MB 752/1 medium (GIBCO) (13), pH 7.2, with 10% (v/v) fetal bovine serum, 10 m*M* Hepes, and 0.09 U/ml low zinc insulin (14) and incubated at 36°C in a humidified 4% CO_2 atmosphere. Within 9–12 days the original cell clumps and 0.1 to 0.5-mm^3 explants formed monolayers composed of multiple cell types, including a flattened layer of epithelial-like immature neuroglial cells, astrocytes, and neuroblasts (13). Neural cell monolayers were used as soon as they were confluent. The medium was changed every other day to maintain pH. Human foreskin fibroblasts, obtained from E. R. Pfefferkorn and used between passages 27–32, were grown in Eagle's minimum essential medium with Earle's salts (GIBCO) and 10% fetal bovine serum until confluent monolayers were produced in 12-well cluster plates. When fibroblasts were compared to neural cells, the medium was changed to complete Waymouth's for infection. Swiss albino 3T3 mouse fibroblasts were grown under similar conditions. Chinese hamster ovary cells were grown in Dulbecco's modified MEM (GIBCO) with 15% fetal bovine serum. Two cloned strains of *T. gondii* were used. The RH strain, obtained from E. R. Pfefferkorn, has been se-

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lected for early death in mice and rapid growth in cell culture over years of laboratory use. The P strain, obtained from L. H. Kasper, is a clone of the M49 strain that grows much more slowly in cell culture than does RH and produces numerous brain cysts in infected mice. Both strains were grown in human fibroblast monolayers; inocula were prepared by freeing the parasites from fibroblasts by forcible passage through a 27-gauge needle. For infections of neural cells several concentrations of newly released *T. gondii* in Waymouth's complete medium were added to replicate fibroblast and neural monolayers, inoculum was replaced with fresh medium after 1 hr and infection was allowed to proceed at 37°C in a humidified atmosphere containing 5% CO_2 for 5 days. The medium was changed 24 and 72 hr after infection to maintain integrity of neural monolayers. Experiments with other cell types were done in a similar fashion using MEM-E and allowing infection to proceed uninterrupted for 4 days. At the completion of the experiment the monolayers were rinsed in phosphate-buffered saline, pH 7.2, fixed in two changes of absolute methanol, dried, and stained in dilute Giemsa (Harleco 620) for 20 min. Stained monolayers were viewed with a Will dissecting microscope and lytic plaques were counted.

Results. Preliminary experiments compared barely confluent Swiss albino 3T3 mouse cells, Chinese hamster ovary (CHO) cells, and human fibroblasts infected by RH strain organisms of equal inocula for yield of organisms at 24 hr and number of plaques at 4 days (Table I). CHO cells grew so rapidly that plaques were not clear. There was no statistical difference in the plaquing efficiency or total parasite yield between human HF cells and the two rodent cell lines. Human fibroblasts produced the most easily read plaques and they were used in the comparison with neural monolayers. The RH strain produced large, easily counted plaques in both fibroblasts and neural monolayers (Figs. 1C and 1D), but the P strain grew slowly, the plaques were smaller (Figs. 1A and 1B), and after 5 days of incubation many infected cells had not lysed and thus parasites had not infected surrounding cells. These older infected cells resembled cysts: They were enlarged and thin walled, and they contained numerous small, darkly stained *T. gondii* (Figs. 1A, inset, and 1B). These cells were counted and added to the plaques to give the number of infected foci in monolayers infected with P strain organisms. In neural and fibroblast monolayers infected by RH strain organisms only fully developed plaques were counted because of the possibility that disturbance of the monolayers associated with media exchange might lead to secondary plaques with this rapidly growing strain.

TABLE I. COMPARISON OF PLAQUING EFFICIENCY AND TOTAL PARASITE YIELD IN THREE HOST CELL TYPES INFECTED BY EQUAL INOCULA OF *T. gondii*

Cell type	Plaques per 100 PFU	Parasite yield
HF	84.3 ± 4.5	3.00 ± 0.2[a]
CHO	ND	2.45 ± 0.7
3T3	85.6 ± 12.6	2.80 ± 0.1

Note. PFU, plaque-forming units; HF, human fibroblasts; ND, not determined.

[a] Total number of organisms per monolayer × 10^6 ± SD.

Table II shows that there is no statistical difference between the number of plaques counted in monolayers of neural cells and fibroblasts inoculated with equal numbers of RH strain organisms. Also, the number of infected foci in neural cells and fibroblasts infected by equal inocula of P strain organisms do not differ significantly. There were noticeable differences between the appearances of monolayers infected by RH and P strain organisms, however. Neural monolayers infected by either strain showed infected immature neuroglia, astrocytes, and neuroblasts, but the plaques formed by lysing cells infected by P strain organisms were small, and cyst-like cells were scattered across the monolayer. These cyst-like cells may represent true cysts in evolution, an intermediate stage between tachyzoites and bradyzoites, or they may be artifactual. Their biological and antigenic characteristics were not assayed in this study. They were most prominent in the immature neuroglia, but similar cells were seen in the human fibroblast monolayers as well. The RH strain did not produce such cells in either type of monolayer, and the plaques produced in both monolayers were much larger than those of P strain organisms for a similar period of incubation. Although plaques were not counted according to cell type, plaques were scattered

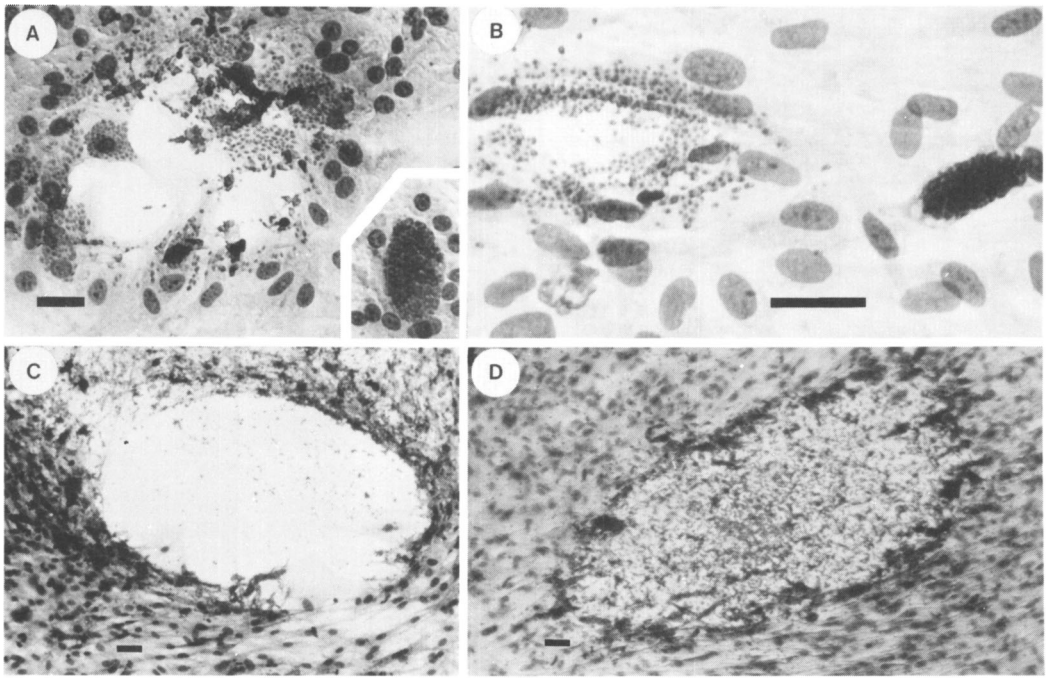

FIG. 1. Infection of cell monolayers by *Toxoplasma gondii*. (A) Neural cell monolayer infected with P strain organisms (inset: cyst-like cell). (B) Fibroblast monolayer infected by P strain. (C) Neural cell monolayer infected with RH strain. (D) Fibroblast monolayer infected by RH strain. Bar = 100 μm.

apparently randomly across the monolayers and neither strain appeared to infect any particular cell type in the neural monolayers preferentially. The flatter neuroglial cells which formed an epithelial-like layer appeared to have better-defined plaques.

Discussion. The relative infectivity of *T. gondii* for various cell types appears to be equivalent. In these studies neither the number of parasites produced per monolayer nor the number of plaque-forming units in a given inoculum differed between rodent and human cells infected by the virulent RH strain. Both the RH and the P strain tachyzoites infected neural monolayers no differently than their respective fibroblast controls. This is not to say that the two strains had equal rates of infection per parasite or that the kinetics of infection were the same between strains or between cell lines. The experimental system was optimized to show a possible difference in infection between cell lines; that none was demonstrated argues that the biological potential of *T. gondii* to infect various host cells is about equal. The real biological difference between parasite strains was obvious from plaque morphology. Even in the absence of immunological factors the P strain formed cyst-like structures, while the RH strain did not.

This study provides no evidence that intrinsic differences between neural and nonneural cells result in an increased susceptibility to infection by *T. gondii*. This suggests that local immunity within the central nervous

TABLE II. NUMBER OF INFECTED FOCI PRODUCED BY A STANDARDIZED INOCULUM OF TWO STRAINS OF *T. gondii* INFECTING MONOLAYERS OF HUMAN FIBROBLASTS OR MOUSE NEURAL CELLS[a]

Strain	Cell type	No.	Foci/monolayer[b] ±SD	*P*[c]
RH	Fibroblast	12	39.3 ± 12.5	0.58
RH	Neural	12	35.6 ± 6.6	
P	Fibroblast	6	32.7 ± 9.3	0.62
P	Neural	6	35.7 ± 8.9	

[a] Two separate experiments, one with each strain of *T. gondii*.

[b] Infected foci and/or plaques counted in Giemsa-stained monolayers.

[c] *P* value determined by Fisher's exact test.

system or other factors that influence the ability of *T. gondii* to encyst or excyst are controlling pathogenic mechanisms, which can be studied *in vivo* without being confounded by differential cell susceptibility.

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