Growth of *Toxoplasma gondii* in Various Tissue Cultures Treated with In·Cn or Interferon[1] (37490)

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Although interference phenomena among viruses, chlamydia or Eperythrozoon and Protozoa (1–4) have been reported, no studies had examined the effect of interferon until Remington and Merigan showed that the cytopathic effect of *Toxoplasma gondii* in tissue culture was inhibited by interferon preparations (5). In another study, toxoplasma eye infection was delayed in rabbits, after treatment with the interferon inducer polyinosinic–polycytidylic acid (In·Cn) (6, 7). The present study was undertaken to determine if In·Cn or interferon itself could modify toxoplasma growth in different types of cell culture including human conjunctival fibroblasts.

Materials and Methods. Free toxoplasma of the RH or Beverly strain obtained by filtration of peritoneal fluid (8) from heavily infected general purpose mice were used throughout this study.

Fibroblast-like cells were grown from human conjunctiva and passaged several times. The 5th to 10th subcultures were used.

Mouse L cells, secondary rabbit kidney cells (RK), human conjunctiva fibroblasts (HC), human U cells (obtained from Dr. K. Cantell, Helsinki) and human embryonic lung cells (WI38) were grown in 30 ml Falcon flasks at 37° in 5% CO_2 atmosphere. Minimal essential medium (MEM) plus 10% fetal bovine serum (FBS) and 1% glutamine supplemented with streptomycin, penicillin, tetracyclin, and mycostatin were used as growth medium. The same medium, but with 2% FBS, was used as maintenance medium.

In·Cn was obtained in a solution of 1000 μg/ml (9) and kept at 4° until used.

1. Growth of Toxoplasma gondii *in tissue cultures.* Monolayers of WI38, HC, RK or L cells grown in 30 ml Falcon flasks were infected with 2×10^6 toxoplasmas/ml of the RH or Beverley strain. After 2 hr adsorption, cultures were washed twice with Hanks' solution. The monolayers were then incubated at 37° in an atmosphere of 5% CO_2 with 5 ml of maintenance medium. Four flasks per group were used to count extracellular toxoplasmas in the medium at 1, 2, 3, and 4 days postinfection.

2. Interferon production in tissue cultures after In·Cn treatment. Interferon was assayed after 24 hr incubation of cell cultures with 100 μg In·Cn. The quantity of human interferon produced by HC and WI38 cells was assayed in human "U" cells by the Sindbis hemagglutination reduction assay (10). Similar assays of fluids from L cells were made using mouse cells and GD-7 virus. Interferon titers in the supernate of RK cells were assayed by the cytopathic effect (CPE) protection method in primary rabbit kidney cultures using vesicular stomatitis virus (VSV) as challenge virus.

3. Effect of In·Cn treatment on the replication of T. gondii. Monolayers of L, RK, WI38, and HC cells were incubated with 1 ml of maintenance medium containing 100 μg of In·Cn. After 3 hr, they were infected with 2×10^6 toxoplasmas/ml of the RH or Beverley strain. Two hours later, cultures

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were washed twice with Hanks' balanced salt solution (HBSS) and 5 ml of maintenance medium with 100 μg In·Cn were added.

The following controls were used: (a) Cultures only infected with 2×10^6 toxoplasmas. (b) Non-infected cultures treated with In·Cn. (c) Cultures neither treated, nor infected.

All cultures were examined daily. At least two cell-culture flasks per group were used to establish the number of toxoplasmas in the fluid medium, at 24, 48, 72 hr postinfection, by counting them in a Neubauer type chamber. The experiment was repeated three times, thus the results represent the average number of extracellular toxoplasma in at least 6 flasks. Statistical evaluation of the results was done by analysis of the variance.

4. Interferon treated cells infected with toxoplasma. Cultures of L and RK cells were incubated with 500 or 200 reference units of mouse serum interferon or 500 or 200 units of rabbit serum interferon (induced with Newcastle disease virus or In·Cn, respectively). The latter contained no toxoplasma antibodies as tested by hemagglutination (11), complement fixation (12), or a simple agglutination test (13). After 3 hr, the cultures were challenged with 2×10^6 *Toxoplasma gondii* of RH or Beverley strain. Two hours later, the cells were washed twice with HBSS and again treated with 500 or 200 units of interferon, each in 5 ml of maintenance medium. Cells were examined daily. Four cell-culture flasks were used to count toxoplasmas in the fluids at 24, 48, and 72 hr postinfection.

Results. 1. Growth of Toxoplasma gondii *in tissue cultures.* It is known that it is easy to cultivate *Toxoplasma gondii* in many types of cells. Figure 1 shows replication in WI38, HC, RK and L cells under our experimental conditions when they were infected with 2×10^6 toxoplasmas. The yield of the parasite (both RH and Beverley strains) reached a peak at the 3rd day postinfection in all tissues. The highest titers were obtained in human conjunctival cells.

2. Interferon production in tissue cultures after In·Cn treatment. Interferon levels in culture fluids of HC or WI38 cells treated with 100 μg In·Cn were essentially similar,

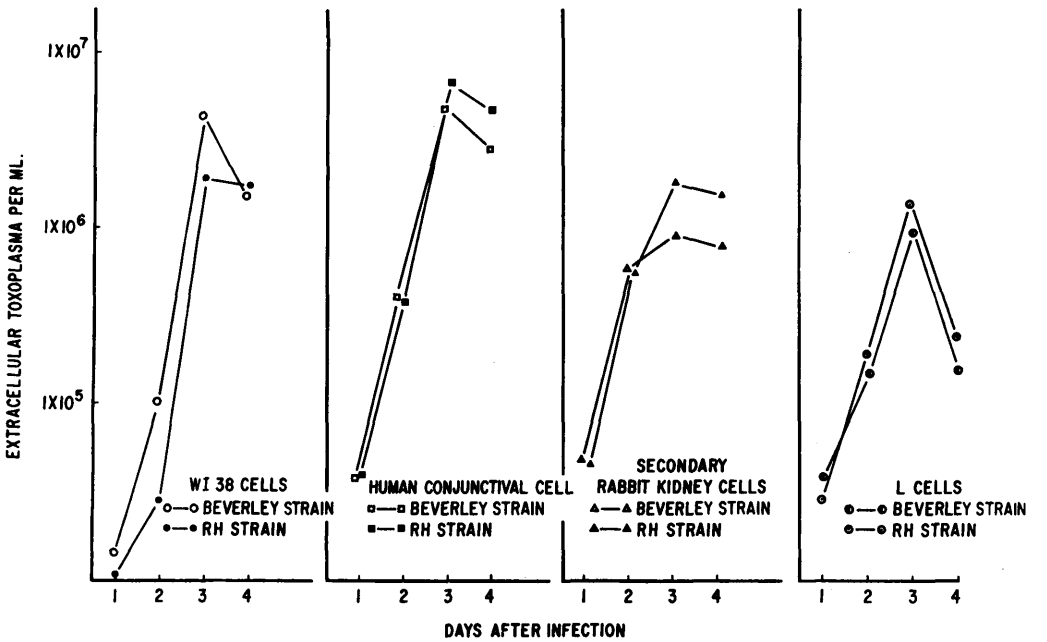

FIG. 1. Growth of *T. gondii* in WI38, Human Conjunctival, Rabbit kidney and L cells, infected with 2×10^6 *T. gondii* organisms, Beverley or RH strain.

decreasing from 50 and 80 units/ml, respectively at the 1st day, to 16 and 30 units at the 3rd day. In RK cells, the interferon levels ranged from 16 to 30 units at 24 hr after In·Cn treatment. Interferon was never detected in L cells treated with In·Cn. As a more sensitive indicator of activation of interferon, we challenged L cells treated with In·Cn with GD-7 virus. There was no inhibition of virus replication.

3. Comparison of kinetics of development of antiviral activity and antitoxoplasma activity in cultures treated with In·Cn. Daily observation of cultures showed no difference in the degree of toxoplasma-induced cytopathology between In·Cn treated or untreated flasks in any of the cell cultures, using 6 replicate cultures per point.

The number of extracellular toxoplasma organisms indicates the number of intracellular parasites (14). Extracellular counts of toxoplasma in the fluid of cultures infected with either RH or Beverley strain, with or without In·Cn treatment were usually negative at 1 day postinfection. After that time, some cells underwent lysis, and trophozoites were liberated into the supernatant fluid. At 48 hr, differences between treated and untreated cultures in the amount of extracellular toxoplasmas were noted but were not statistically significant. Statistically significant differences ($p = 0.001$) were found at 72 hr postinfection with both toxoplasma strains in L, RK, HC and WI38 cells (Fig. 2).

In order to determine whether 24-hr treatment of cells with 100 μg In·Cn affected viral multiplication, HC and W138 cells were challenged with a high multiplicity of Sindbis virus, mouse L cells were challenged with GD-7 virus, and RK cells were challenged with VSV (10). The viral yields in treated and untreated cultures were determined by hemagglutination assay for Sindbis and GD-7 viruses, and by plaque assay in chick embryo cells for VSV. The degree of inhibition of viral replication in treated HC, WI38, and RK cells were 2.0 $\log_{10}$ (as expected from earlier studies which demonstrated maximal induction of the interferon system prior to 24 hr) (9). No In·Cn induced inhibition of virus yield in mouse L cells was detected (15). These findings indicate a dissociation between In·Cn induced inhibition of viruses and *T. gondii,* both with regard to (a) time of development of resistance in all cell strains examined and (b) absence of antiviral activity but presence of resistance to *T. gondii* in In·Cn treated L cells.

4. Effect of interferon treatment during T. gondii *infection of cell cultures.* In RK cultures incubated with 500 units of interferon and infected with the RH or Beverley toxoplasma strains, a slight but significant decrease of the cytopathic effect was observed as compared with untreated controls, starting

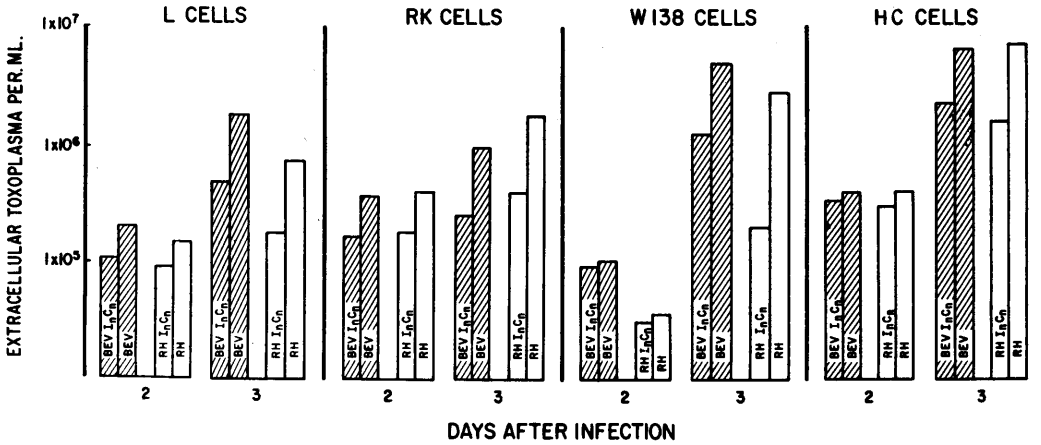

FIG. 2. Number of extracellular *Toxoplasma gondii,* Beverley or RH strain, in the supernatant fluid of L, RK, WI38 and HC cells, treated with 100 μg of In·Cn and untreated controls.

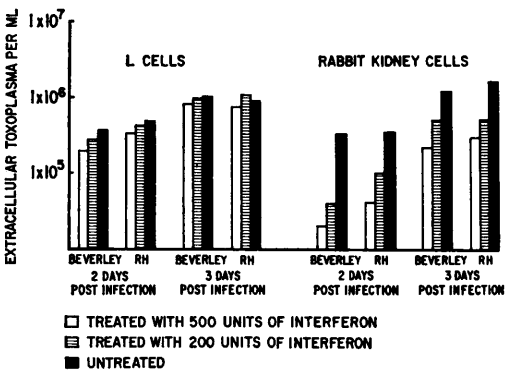

FIG. 3. Number of extracellular toxoplasma of the Beverley or RH strain in the supernatant fluid of infected L cells and rabbit kidney cells, treated with 500 or 200 units of mouse or rabbit interferon respectively, compared with untreated controls.

with day 1 postinfection. No such difference was observed when 200 units of interferon were applied. L cells treated with 500 or 200 international reference units of interferon were not protected against the cytopathic effect exerted by toxoplasma.

The number of extracellular parasites was significantly decreased ($p = 0.05$) at 48 and 72 hr postinfection in RK cells treated with 500 or 200 units of interferon, the decrease being greater in those cultures treated with 500 units.

In L cells, interferon treatment (200 or 500 units) had no significant effect upon the extracellular toxoplasma counts; (Fig. 3) however, this concentration of interferon inhibited the replication of GD-7 virus by 3 $\log_{10}$.

Discussion. The smaller number of extracellular toxoplasma organisms obtained from In·Cn treated cells at 72 hr postinfection indicates that the replication of the protozoon could be diminished by this treatment. It is not clear however, whether the alteration of the host–parasite relationship was due to the interferon activity induced by the treatment, or by some other action of In·Cn. For example, in our experiments, HC, RK, and WI38 cells produced interferon within 24 hr of In·Cn treatment, but inhibition of toxoplasma was not apparent until 72 hr. Also, L cells in which toxoplasma was inhibited by In·Cn did not produce interferon nor did they develop the antiviral state after treatment with In·Cn in the absence of DE AE dextran (15). Thus, this effect of In·Cn on toxoplasma is probably due to a noninterferon action. The report that semisynthetic derivatives of Rifamycin inhibited toxoplasma multiplication in L cells (16) suggested that toxic effects upon cells could suppress the growth of toxoplasma.

The results of the experiments employing interferon support this theory. Although 500 or 200 units of rabbit interferon decreased the effect of toxoplasma infection in RK cells, as measured by CPE activity and by extracellular number of toxoplasmas obtained from the culture, no effect was obtained by either method of assay in L cells incubated with the same amount of mouse interferon. Since pure interferon is not available, the observed inhibition in the rabbit system could conceivably be due to other material in the interferon preparation. Our finding of the lack of inhibition in L cells differs from the observation of Remington and Merigan, who reported that L cells were protected against toxoplasma infection by interferon (5). Their experimental design was different in that they used a much smaller challenge dose of *T. gondii*. Since, in our experiment a high challenge dose applied to RK resulted in inhibition, but the same dose in L cells did not, it does not seem likely that this difference in the result was due to difference in challenge dose.

These results strongly suggest that the interferon system may not affect toxoplasma. Further studies in this area will help elucidate this problem.

Summary. The present study was undertaken to determine whether interferon or an interferon inducer, polyinosinic–polycytidylic acid (In·Cn) could modify the growth of *T. gondii* (RH and Beverley strain) in several different types of cell culture. Pretreatment of human WI38, conjunctiva, rabbit kidney or mouse L cells with In·Cn induced interferon within 24 hr in all but the L cells. Inhibition of toxoplasma multiplication occurred in all the treated cell types including the L cells, but the inhibition did not begin until 72 hr. These findings suggest a noninterferon

mechanism for inhibition of toxoplasma by In·Cn. In other experiments a crude rabbit interferon preparation inhibited toxoplasma replication in rabbit kidney cells, but a similar mouse interferon preparation failed to inhibit toxoplasma multiplication in mouse L cells. In both cell types the interferon exerted a strong inhibitory effect on virus replication. Thus interferon preparations did not consistently inhibit *T. gondii*.

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