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Mechanisms of Interferon-Induced Inhibition of *Toxoplasma gondii* Replication in Human Retinal Pigment Epithelial Cells

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Inflammation associated with retinochoroiditis is a major complication of ocular toxoplasmosis in infants and immunocompetent individuals. Moreover, *Toxoplasma gondii*-induced retinal disease causes serious complications in patients with AIDS and transplant patients. The retinal pigment epithelial (RPE) cell is an important regulatory cell within the retina and is one of the cells infected with *T. gondii* in in vivo. We have developed a human RPE (HRPE) cell in vitro model system to evaluate *T. gondii* replication and the regulation of this replication by cytokines. *T. gondii* replication was quantitated by counting the foci of infection (plaque formation) and the numbers of tachyzoites released into the supernatant fluids. Pretreatment of cultures with recombinant human tumor necrosis factor alpha, alpha interferon (IFN-α), IFN-β, or IFN-γ for 24 h prior to inoculation inhibited *T. gondii* replication in a dose-dependent manner. Of these cytokines, IFN-γ was the most potent, and *T. gondii* replication was completely inhibited at a concentration of 100 U/ml. The anti-toxoplasmotic activity of IFN-γ was significantly blocked by monoclonal antibody to IFN-γ. Treatment of the cultures with IFN-γ from day 1 or 2 postinoculation with *T. gondii* also offered protection against the parasite. The anti-toxoplasmotic activity of tumor necrosis factor alpha or IFN-α, -β, or -γ in these cultures was found to be independent of the nitric oxide (NO) pathway, since NO production was not found in HRPE cells treated with these cytokines. However, addition of tryptophan to IFN-γ-treated cells significantly reversed the inhibitory effects of IFN-γ, suggesting that IFN-γ acts by depleting cellular tryptophan. This effect was further confirmed by reverse transcription-PCR and Northern (RNA) blot analysis, which indicated induction of indoleamine 2,3-dioxygenase (IDO), an enzyme that converts tryptophan to kynurenine. These results indicated that interferons inhibited *T. gondii* replication in HRPE by NO-independent but IDO-dependent mechanisms. This in vitro model of *T. gondii* replication in HRPE may be useful in evaluating the effects of cytokines and drugs on *T. gondii* replication within the retina.

Toxoplasma gondii, an obligate intracellular protozoan parasite, infects a large population of humans throughout the world, as shown by the prevalent presence of anti-toxoplasma antibodies in the serum (11, 34, 38). In normal individuals, *T. gondii* infection results in asymptomatic infections and then persistent infections which are usually controlled by the host immune system. However, in immunocompromised hosts, such as AIDS patients and organ transplant recipients, the dormant parasite becomes activated, causing life-threatening diseases (15, 21, 33, 34, 48). Encephalitis and retinochoroiditis, resulting from the activation of dormant tissue cysts, are the most serious complications of toxoplasmosis (15, 16, 21, 48).

Ocular toxoplasmosis is one of the common causes of uveitis, accounting for 10 to 50% of cases, depending on the geographic location (16, 18, 20, 34, 38, 52). The majority of ocular toxoplasmosis is the result of congenital infection (34, 38, 52). However, recent evidence suggests that acquired toxoplasmosis is also a cause of retinochoroiditis (47). The primary target of ocular toxoplasmosis is the neural retina, where the parasite forms intracellular cysts (16, 17, 33, 51, 52). The presence of free tachyzoites and cysts within retinal pigment epithelium (RPE) cells was also observed (16, 33). Ocular toxoplasmosis involves necrosis of the neural retina and the RPE and subretinal and choroidal neovascularization, as well as focal inflammation (16, 17, 33, 51, 52). The inflammatory processes associated with retinal infection of *T. gondii* may cause damage to the Bruch's membrane, resulting in the disruption of the choroidoretinal interface (33, 38, 51, 52).

Several studies have indicated the role of various cytokines in the regulation of *T. gondii* infection in animal models (5, 17, 23, 26, 35, 49, 50). Administration of recombinant murine gamma interferon (IFN-γ) to outbred Swiss Webster mice provided complete protection against infection by C56, a virulent strain of *T. gondii* (26). In BALB/c mice, prior treatment with anti-IFN-γ monoclonal antibody (MAb) before inoculation with *T. gondii* (ME 49 [avirulant strain]) resulted in 100% mortality (49, 50). Significant increase in the levels of IFN-γ in the serum and peritoneal fluid were observed in mice infected with *T. gondii* (3, 45). Differences in the levels of IFN-γ in the serum of *T. gondii*-infected resistant (C57BL/6) and susceptible (BALB/c) mice strains (3, 45) suggest a protective role for IFN-γ.

The replication of *T. gondii* in cultures of primary and established cell lines has been investigated (6, 7, 22, 25, 31, 36, 39–42, 44, 53). In human fibroblast cells, the replication of *T. gondii* was reported to be inhibited by recombinant IFN-γ but not by IFN-α or IFN-β (42). Recombinant IFN-α and IFN-β are shown to exhibit anti-taxoplasmotic activity in human macrophages, although to a lesser extent than IFN-γ (53). In human microglial cells and astrocytes, IFN-γ in combination with lipopolysaccharide (LPS) or interleukin-1 (IL-1) was shown to

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exhibit inhibitory effects on *T. gondii* replication (7, 39). These observations suggest that the effects of interferons and cytokines on intracellular multiplication of *T. gondii* in in vitro systems appear to be dependent on cell types.

RPE, a single layer of epithelium present between the choroid and neuroretina, plays a vital role in inflammatory and infectious diseases of the posterior pole of the eye (54). RPE is known to secrete IL-6 (29), IL-8 (13), intercellular adhesion molecule 1 (30), and nitric oxide (NO) (24) in response to stimulation by inflammatory cytokines and LPS. In addition, in the presence of inflammatory mediators, RPE upregulates cell surface expression of major histocompatibility complex (MHC) class I and major histocompatibility complex class II molecules and participates in the antigen presentation process and immunoregulation within the eye (10, 37). Tachyzoites and cysts of *T. gondii* are reported to be present in the neuroretinas and RPEs of ocular toxoplasmosis patients (16, 33). The patterns of *T. gondii* replication within RPE cells may reflect in vivo conditions and offer a useful model to evaluate the factors that regulate the retinal infection. Therefore, we have studied the replication of *T. gondii* in human RPE (HRPE) cultures and evaluated the roles and mechanisms of action of interferons and other cytokines as anti-toxoplasmosis agents. In this report, we demonstrate that interferons inhibit *T. gondii* replication in the HRPE by the induction of indoleamine 2,3-dioxygenase (IDO), an enzyme that degrades tryptophan to kynurenine, but not by NO production.

MATERIALS AND METHODS

Materials. Human recombinant IFN-γ (specific activity, 2×10^7 U/mg) and tumor necrosis factor alpha (TNF-α) (specific activity, 10^8 U/mg) were purchased from Boehringer Mannheim, (Indianapolis, Ind.). Human recombinant IFN-α, subtype 2B (specific activity, 3×10^8 U/mg), was purchased from Collaborative Biomedical Products (Bedford, Mass.). Human recombinant fibroblast IFN-β (specific activity, 10^7 U/ml) was obtained from Green Cross (Osaka, Japan). Human recombinant IL-1β was purchased from R&D Systems (Minneapolis, Minn.). Monoclonal mouse anti-human IFN-γ was obtained from Genzyme (Cambridge, Mass.). Monoclonal mouse anti-*T. gondii* RH and rabbit polyclonal anti-*T. gondii* RH were purchased from Biogenex Laboratories (San Ramon, Calif.) and Virostat (Portland, Maine), respectively. The Diff-Quik stain kit was bought from Baxter (Mcgaw Park, Ill.). L-Tryptophan, L-kynurenine, L-arginine, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.).

HRPE cultures. Primary HRPE cell lines were established from donor eyes as described previously (29). These cell lines form monolayers characteristic of epithelial cells. The homogeneity of the cell population was further confirmed by positive immunostaining with MAbs to cytokeratins (29). Contamination of cells with fibroblasts or endothelial cells was excluded, since 100% of the cells exhibited the presence of cytokeratin in these cells. HRPE cultures at passages 6 to 13 were used in all of the experiments reported in this study.

Preparation of *T. gondii* RH. *T. gondii* RH cells obtained from R. Gazzinelli, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, were grown in HRPE cultures and were frozen in liquid nitrogen. Frozen stocks of *T. gondii* were propagated in HRPE cultures once prior to use in these studies. When approximately 70% of the cells were infected with the parasite, supernatant fluids were collected carefully with minimal dispersal of the cells. Supernatant fluids were initially centrifuged at 200 rpm (RC5C [Sorvall]) for 5 min to sediment contaminating cells and debris. The supernatant fluids were further centrifuged at 2,000 rpm for 20 min to pellet the parasites. *T. gondii* cells were resuspended in serum-free medium (SFM) and were centrifuged again. *T. gondii* cells were counted and suspended in SFM at a concentration of 2×10^6 tachyzoites per ml and were used for the inoculation studies.

Infection of HRPE cells with *T. gondii*. HRPE cells were grown to confluence in eight-well glass chamber slides (Nunc, Naperville, Ill.) in minimum essential medium supplemented with 10% fetal bovine serum, nonessential amino acids, and an antibiotic-antimycotic mixture. The cultures were washed with SFM twice, and 400 μl of SFM was added to each well. The cultures were incubated with tachyzoites of *T. gondii* (10^5 in 50 μl of SFM) for 2 h at 37°C. Then, the cultures were washed twice with SFM to remove nonadherent parasites and were incubated in SFM. After different time points, supernatant fluids were collected and cultures were fixed and stained with the Diff-Quick stain kit. The replication of *T. gondii* in HRPE cultures was evaluated by counting the numbers of foci of infection (plaques) in the cultures and the tachyzoites released into the supernatant fluids.

Indirect immunofluorescence detection of *T. gondii*. HRPE cultures grown to confluence in eight-well chamber slides were infected with tachyzoites of *T. gondii* as described above. At different time points, the cultures were fixed for 10 min in an acetone-methanol mixture (1:1) precooled to −20°C. The slides were air dried and stored until they were used for staining. The cells were incubated with either mouse MAb (5 μg of immunoglobulin G [IgG]/ml) or rabbit polyclonal antibody (100 μg of IgG/ml) for 1 h at 37°C in a humidified chamber. After the cells were washed three times in phosphate-buffered saline (PBS), they were incubated with fluorescein isothiocyanate-conjugated horse anti-mouse IgG or goat anti-rabbit IgG, respectively, for 1 h at 37°C. The cells were washed again three times with PBS, mounted with Aqua-mount (Lerner Laboratories, Pittsburgh, Pa.), and viewed under a fluorescence microscope.

Effects of cytokines on *T. gondii* replication. HRPE cultures grown to confluence in eight-well chamber slides were washed with SFM twice and incubated in SFM (400 μl per well) containing various concentrations of TNF-α, IFN-α, -β, or -γ, or other cytokines. After 24 h, 10^5 tachyzoites of *T. gondii* suspended in 50 μl of SFM were added to each well. After a 2-h adsorption period at 37°C, the cultures were washed twice with SFM to remove cytokines and nonadherent parasites and were further incubated in cytokine free SFM. At the indicated times, media were collected and cultures were fixed and stained for the evaluation of *T. gondii* replication as described above. We did not observe any changes in the gross morphology of the HRPE cells upon treatment with the cytokines described above at the indicated concentrations.

Determination of NO production. The cultures were incubated in phenol red-free SFM in the presence of various cytokines. At the indicated times, the media were harvested and nitrite levels, which reflect NO production by the cells, were estimated (24) with a Griess reagent. A 100-μl volume of medium was mixed with 100 μl of a Griess reagent (a 1:1 mixture of a 1% solution of sulfanilamide in 5% phosphoric acid and a 0.1% aqueous solution of naphthylethylenediamine hydrochloride), and the color developed was read at 540 nm. Nitrite levels were determined from the standard graph prepared with sodium nitrite.

Effects of L-tryptophan on IFN-γ inhibition of *T. gondii* replication. HRPE cultures grown in eight-well chamber slides were washed with SFM twice and were incubated in SFM containing various combinations of L-tryptophan (0, 20, 50, or 100 μg/ml) and IFN-γ (0, 5, 10, 20, and 40 U/ml). After 24 h, tachyzoites of *T. gondii* (10^5 in 50 μl of SFM) were added to each well. After 2 h of incubation at 37°C, the media were removed, and the cells were washed twice with SFM and were further incubated in SFM containing original tryptophan concentrations but without IFN-γ. After days 1, 2, 3, and 4, tryptophan was added to each well to give a final concentration of 20, 50, or 100 μg/ml. This was done to replenish the tryptophan levels that might have been depleted by IDO-induced by IFN-γ. Media were collected and cultures were fixed after 5 days to count *T. gondii* cells and plaque formation. In separate experiments, the effects of L-kynurenine (100 μg/ml) were also tested in the presence and absence of IFN-γ.

Analysis of IDO expression by reverse transcription-PCR. HRPE cultures grown to confluence in 60-mm dishes were treated with medium or medium containing TNF-α (10 ng/ml), IFN-α (1,000 U/ml), IFN-β (1,000 U/ml), or IFN-γ (100 U/ml). After 24 h of cytokine treatment, total RNA was prepared from one set of cultures. The three other sets of cultures, prepared at the same time and treated as described above, were washed twice to remove the cytokines and were further incubated in SFM. After 24, 48, and 72 h, RNA was prepared from post-cytokine-treated HRPE cultures. Primers ACA GAC CAC AAG TCA CAG CG (sense) and AAC TGA GCA GCA TGT CCT CC (antisense) were designed from the cDNA sequence reported for the human IDO (8). Total RNA prepared by using RNA Zol (Tel test, Friends Wood, Tex.) was reverse transcribed with cDNA cycle kit (Invitrogen, San Diego, Calif.) and used as template for PCR amplifications. The PCR mixture (50 μl) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, a cDNA preparation (corresponding to 50 ng of RNA), 1 μM each primer, 200 μM each of the deoxynucleoside triphosphates, and 5 U of *Taq* polymerase (Perkin-Elmer Cetus, Norfolk, Conn.). PCR amplification was performed by subjecting the mixture to 35 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 1 min. The reaction mixture was kept at 72°C for 10 min after PCR cycles were completed. An aliquot (20 μl) of the sample was subjected to agarose gel electrophoresis, stained with ethidium bromide, and photographed under UV light. The 662-bp PCR product detected in IFN-γ-treated samples (data not shown) was excised from the agarose gel and subcloned into pCR II TA cloning Vector (Invitrogen). The identity of the product with the reported cDNA sequence was confirmed by sequencing the subclones by dideoxynucleotide chain termination with Sequenase version 2.0 (U.S. Biochemicals, Cleveland, Ohio).

Northern (RNA) blot analysis. Total cellular RNA from HRPE cultures treated with cytokines was prepared as described above. The subcloned IDO PCR product of 662 bp was used as a probe for Northern blot analysis. A human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe was generated by PCR with gene-specific primers. RNA was fractionated by 1.1% agarose gel electrophoresis in the presence of formaldehyde, transferred to Genescreen nylon membranes (NEN Dupont, Boston, Mass.), and immobilized by UV cross-linking. The membranes were prehybridized at 42°C for 4 h in Hybrisol I con-

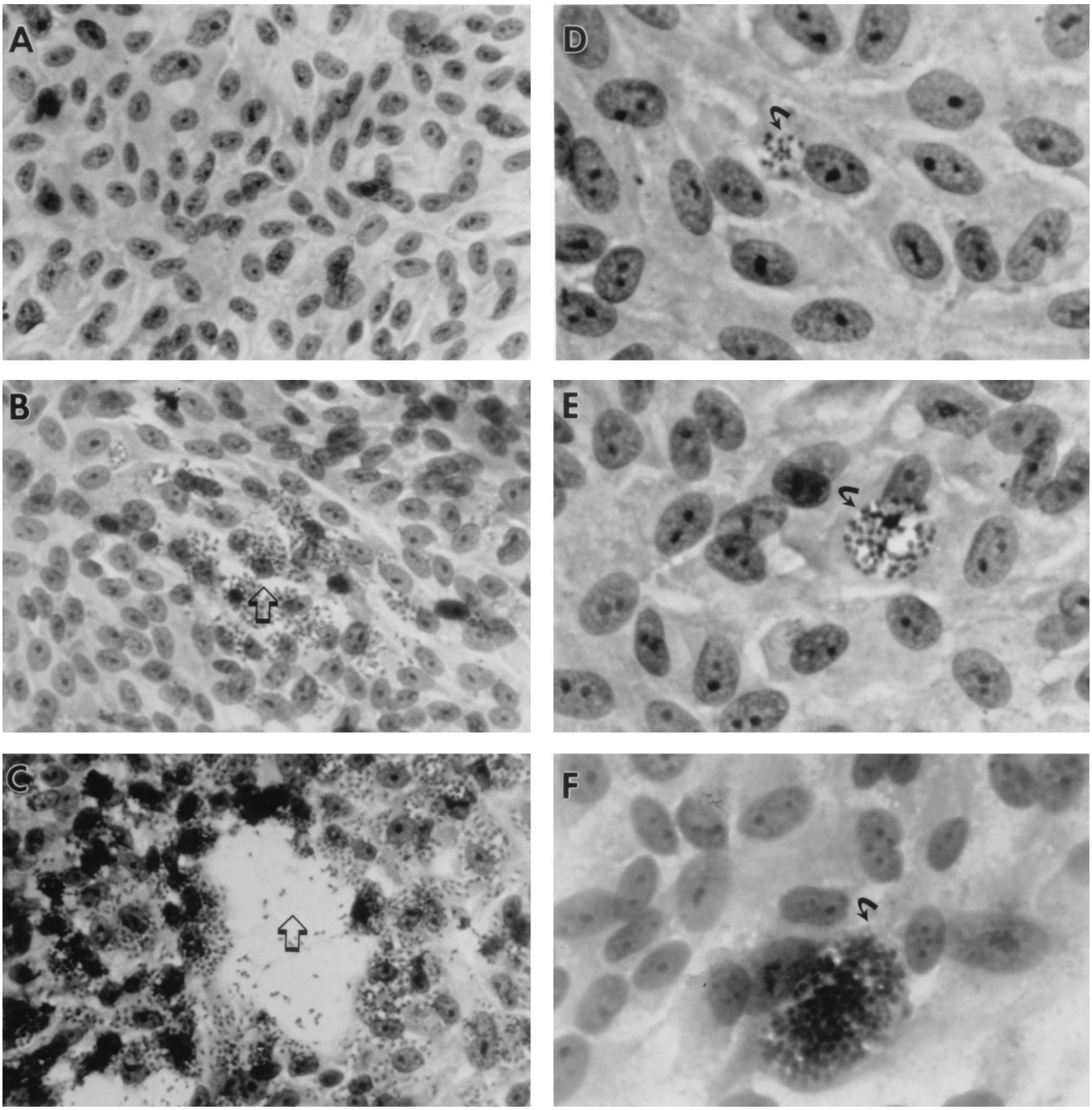

FIG. 1. *T. gondii* replication in HRPE cultures. Representative photomicrographs illustrate parasite replication and plaque and cyst-like formation in the cultures. The cultures were inoculated with tachyzoites of *T. gondii*. On different days postinoculation, cultures were fixed and stained with the Diff-Quick stain kit. (A) Uninfected cells; (B) parasite replication and invasion of neighboring cells in one focal area 3 to 4 days postinoculation; (C) host cell destruction and plaque formation with the release of parasites into the medium 4 to 5 days postinoculation; (D) parasites enclosed in a vacuole inside the cell cytoplasm 1 to 2 days postinoculation; (E) parasites dividing and forming aggregates within the vacuole 2 to 4 days postinoculation; (F) fully formed cyst-like structure of *T. gondii* 3 to 5 days postinoculation. Arrows, locations of plaques and cyst in the cultures. Magnifications, ×200 (A, B, and C) and ×400 (D, E, and F).

taining 50% formamide (Oncor, Gaithersburg, Md.) and then hybridized overnight at 42°C in a similar hybridization solution containing the cDNA probe labeled with ^{32}P by random priming. The membranes were washed under stringent conditions and were exposed to Kodak XAR film. The same membranes were stripped completely and reprobed with a labeled GAPDH cDNA probe. Northern blots of IDO and GAPDH were subjected to densitometric analysis with the image analysis system, version 2.1 (model GS-700 [Bio-Rad]), and the level of IDO induction was expressed relative to the levels of GAPDH.

RESULTS

Characterization of *T. gondii* replication in HRPE cells. HRPE cultures were infected with *T. gondii* tachyzoites at a multiplicity of infection of 1. After a 2-h adsorption period, the nonadherent parasites were removed and the cells were washed twice, refed with media, and incubated at 37°C. Para-

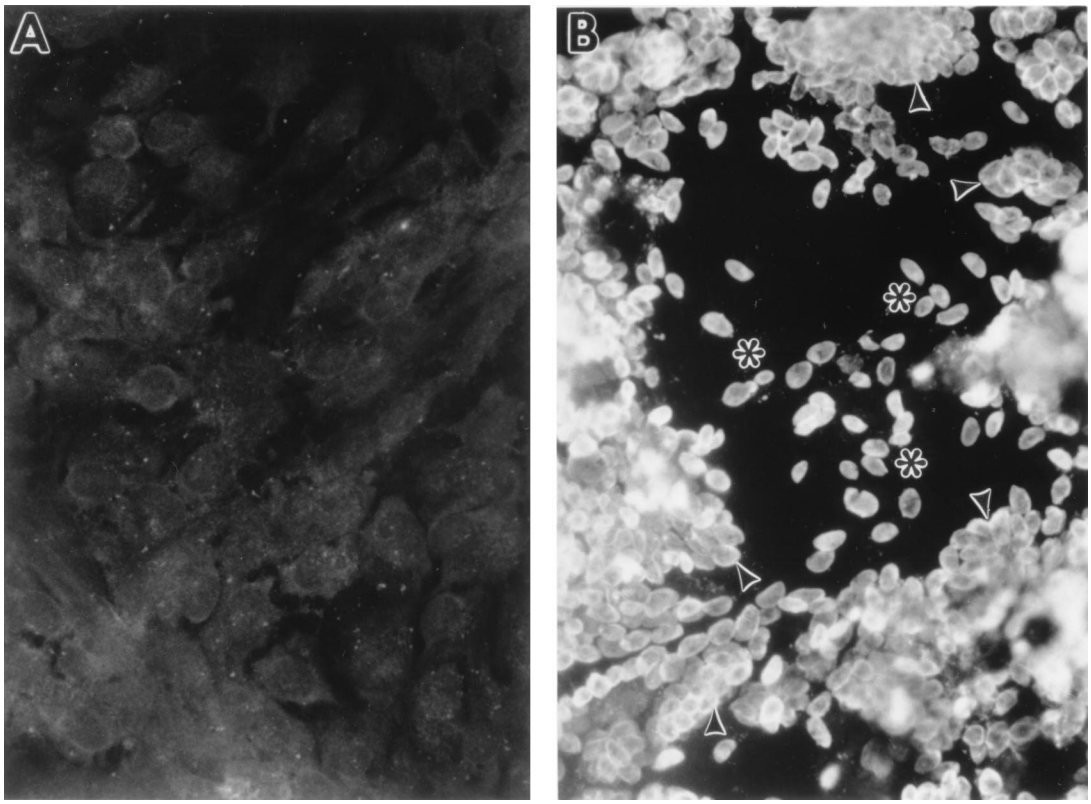

FIG. 2. Immunofluorescence detection of *T. gondii* in infected HRPE cells. Cultures were washed, fixed, and stained with MAb raised against *T. gondii* RH as described in Materials Methods. This MAb recognizes a 30-kDa membrane antigen of the tachyzoite of *T. gondii*. Fluorescent images of uninfected (A) and *T. gondii*-infected (B) HRPE cells shown are at the same magnification (×400). Asterisks and arrowheads in panel B, presence of free tachyzoites in the plaques and clusters of tachyzoites present intracellularly within the intact infected cells, respectively.

site multiplication was observed within the cells after 2 days of incubation (Fig. 1B). Discrete areas of *T. gondii* replication in the host cells were identified as foci of infection or plaques (Fig. 1B and C). The number of tachyzoites increased, resulting in cell rupture and release of parasites into the supernatant fluid and subsequent invasion of the neighboring cells. This cycle of intracellular multiplication of the parasite, destruction of the host cell, and invasion of the surrounding cells continued until the entire HRPE monolayer was destroyed.

In some of the infected HRPE cells, tachyzoites appeared to be enclosed in a vacuole resembling a parasitophorous vacuole in the host cytoplasm (Fig. 1D). Inside the vacuole, tachyzoites divided, forming cyst-like structures containing more than 100 parasites (Fig. 1E and F). Occasionally, these types of cysts were seen in HRPE cultures. The nature and conditions which influence the formation of these cyst-like structures in the HRPE are not known. We have not observed a correlation between the formation of cyst-like structures and the treatment of cultures with interferons and cytokines.

Detection of parasite with *T. gondii*-specific antibodies. Indirect immunofluorescence staining of the cultures, with MAbs and polyclonal antibodies raised against the RH strain of *T. gondii*, was performed to confirm the localization of the parasite. MAb clone G11 (Biogenex catalog no. MU125-UC), which recognizes a tachyzoite membrane antigen of 30 kDa, was found to react with the parasite. We used this antibody for the detection of tachyzoites. Intense staining of the parasites present in the plaques and within the cytoplasmic compartment of the infected cells was observed (Fig. 2B). In contrast, no reaction was noticed in uninfected cells (Fig. 2A). Intracellular cyst-like structures also reacted strongly with this MAb (data not shown). These data indicate that the cyst-like structures still retain tachyzoite antigens. Since bradyzoite specific antibodies are not presently available to us, it is not possible to determine if these cyst-like structures were also beginning to express bradyzoite antigens.

Comparison of plaque formation and parasites released from HRPE cells infected with *T. gondii*. *T. gondii* replication was measured by two methods, by counting the number of foci of infection (plaques) in HRPE cultures and by counting the number of parasites released from the cells into the supernatant fluids. A comparison of the number of plaques and tachyzoites released is shown in Fig. 3. On the day after inoculation, no plaques or free tachyzoites were detected in the cultures. Small plaques were first observed on day 2 postinoculation. The size and the number of plaques increased with time. After day 5, the plaques increased in size and covered most of the area of the cultures. Therefore, plaque counts were accurately evaluated up to day 5 in the replication cycle. Tachyzoites were first observed in the supernatant fluid on day 4 and increased in number on day 5 and 6. These studies demonstrated that *T. gondii* replication in RPE cells can be quantitated by counting plaques at days 3 to 5 and by counting the tachyzoites released into the supernatant fluid at day 4 or 5.

Effect of cytokines on *T. gondii* replication in HRPE cells. Previous in vitro studies of *T. gondii* infections have used 24-, 48-, or 72-h treatment of the cells with cytokines before inoculation with the parasite (7, 36, 39–42, 53). Preliminary experiments with HRPE cells have shown that a 24-h preincubation

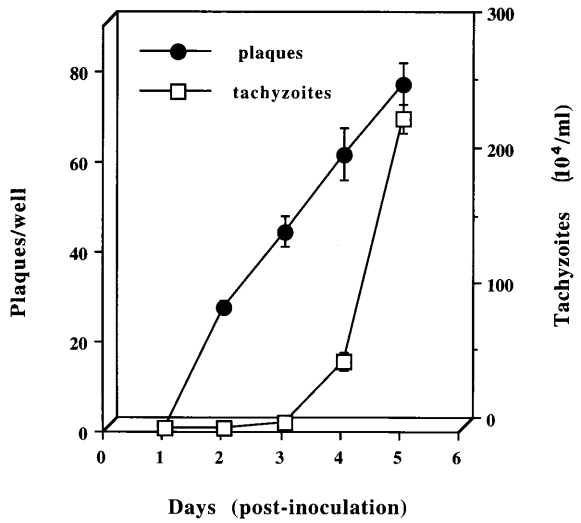

FIG. 3. Time course of plaque formation and release of tachyzoites from HRPE cultures. Cultures grown in eight-well chamber slides were inoculated with tachyzoites of *T. gondii* as described in Materials and Methods. At days 1 to 5 postinoculation, supernatant fluids were collected and cultures were fixed and stained with the Diff-Quick stain kit. The numbers of plaques formed per well and the numbers of parasites released into the supernatant fluids were counted. The results are the means ± standard errors of the means for four experiments.

with the cytokines offers effective inhibition of *T. gondii* replication. Therefore, the effects of various cytokines on *T. gondii* replication were studied by incubating cells with these agents 24 h prior to inoculation with the parasite. Pretreatment of the cells with IL-1α, IL-1β, IL-6, or LPS had no significant effect on *T. gondii* replication (data not shown). In contrast, TNF-α, IFN-α, IFN-β, and IFN-γ inhibited *T. gondii* replication in HRPE cells in a dose-dependent manner (Fig. 4). TNF-α (10 ng/ml), IFN-α (1,000 U/ml), IFN-β (1,000 U/ml), and IFN-γ (100 U/ml) inhibited *T. gondii* replication by 49.3, 57.1, 72.9, and 100%, respectively (P, <0.001). IFN-α, IFN-β, and IFN-γ

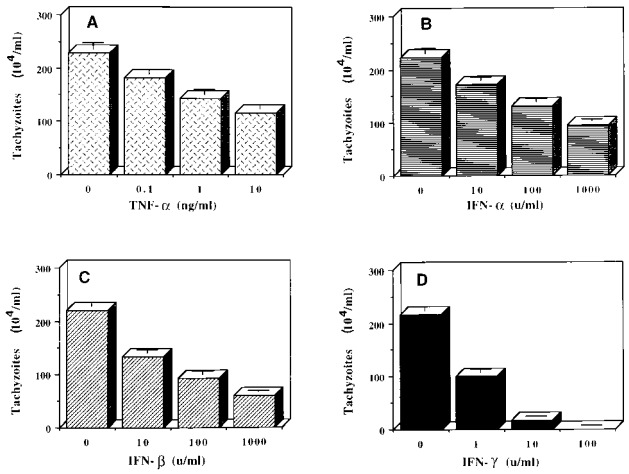

FIG. 4. Effects of recombinant TNF-α (A), IFN-α (B), IFN-β (C), and IFN-γ (D) on *T. gondii* replication in HRPE cultures. Cultures were treated for 24 h with the indicated concentrations of cytokine prior to inoculation with the parasite. After inoculation of the cells with the parasite, the cultures were not treated with the cytokines. Five days after inoculation, the cultures were rinsed twice and supernatant fluids were collected. *T. gondii* replication was quantitated by counting the numbers of tachyzoites released into the supernatant fluids. The results are the means ± standard errors of the means for four experiments.

TABLE 1. Effect of IFN-γ treatments on *T. gondii* replication in HRPE cells

Treatment[a]	Time of exposure (h)	No. of foci of infection (plaques/well)[b]	No. of *T. gondii* cells released (10^4/well)[b]
None		98.62 ± 4.37	157.12 ± 7.12
IFN-γ	−24–0	0.00	0.00
IFN-γ	0–120	0.00	0.00
IFN-γ	24–120	0.00	3.60 ± 1.43
IFN-γ	48–120	20.62 ± 4.68	29.87 ± 6.92
IFN-γ	72–120	69.25 ± 7.29	115.22 ± 5.24

[a] The concentration of IFN-γ in all of the treatments was 100 U/ml.
[b] The numbers of foci of infection (plaques) in the cultures and of *T. gondii* released into the supernatant fluids were determined 5 days after parasite inoculation. Results are the means ± standard errors of the means for eight observations.

at a concentration of 10 U/ml inhibited *T. gondii* replication in HRPE cultures by 22.3, 39.3, and 92.3%, respectively. These results indicated that IFN-γ was the most potent inhibitor of *T. gondii* replication in HRPE cells. The differences in the inhibition of *T. gondii* replication between 1.0 and 10.0 ng of TNF-α per ml were not statistically significant (P, 0.084). The differences in the inhibition of replication of *T. gondii* between all other samples of TNF-α, IFN-α, and IFN-β were significant (P, <0.01 [Student t test]). Inhibition of replication of *T. gondii* was highly significant (P, <0.0001) between all IFN-γ concentrations tested.

Inhibition of IFN-γ anti-toxoplasma activity by neutralizing MAb. The specificity of inhibition of *T. gondii* replication by IFN-γ was evaluated with neutralizing antibody to IFN-γ. IFN-γ (100 U/ml), IFN-γ (100 U/ml) plus anti-IFN-γ MAb, or anti-IFN-γ MAb were incubated for 2 h at 37°C. These samples were then added to RPE cell cultures. After a 24-h incubation period, the cells were infected with *T. gondii*. After a 2-h adsorption period, inoculum was removed, and the cultures were washed twice, refed with fresh media, and incubated at 37°C. *T. gondii* replication was quantitated by both the plaque assay and the release of tachyzoites into the supernatant fluid. IFN-γ alone completely inhibited *T. gondii* replication. When IFN-γ was pretreated with anti-IFN-γ MAb, the ability of IFN-γ to block *T. gondii* replication was significantly (P, <0.001) inhibited (data not shown). In contrast, anti-IFN-γ MAb alone did not affect the *T. gondii* replication.

Kinetics of IFN-γ treatment on *T. gondii* replication. In all of the previous studies of *T. gondii* infections in vitro (7, 36, 39–42, 53), the cells were treated for 24, 48, or 72 h with the cytokines prior to infection. No postinfection cytokine treatments or comparison of the effects of pre- and postinfection cytokine treatments on anti-toxoplasma activities were made in these studies. We wanted to evaluate whether treatments with the cytokines at different stages postinfection offer protection against *T. gondii*. IFN-γ was selected for this study because of its potent anti-toxoplasmotic activity. HRPE cultures incubated with IFN-γ either before or after inoculation with *T. gondii* were examined for parasite replication (Table 1). Pretreatment of cultures with IFN-γ (100 U/ml) for 1 day before inoculation (−24 to 0 h) completely abolished *T. gondii* replication. Likewise, when IFN-γ was added at the same time (0 to 120 h) or 1 day after (24 to 120 h) *T. gondii* inoculation, the replication of the parasite was completely inhibited. The effectiveness of IFN-γ as an anti-toxoplasma agent decreased progressively when IFN-γ was added after 2 or 3 days of inoculation with the parasite (Table 1). Moreover, treatment of the

cultures with IFN-γ after 4 days of parasite inoculation was totally ineffective in controlling *T. gondii* replication (data not shown). The results of these experiments indicate that IFN-γ may act as an anti-toxoplasma agent by directly affecting parasite and/or host cell metabolic pathways within HRPE cells.

Inhibition of *T. gondii* replication in HRPE by cytokines is not dependent on NO production. It has been reported by others (1, 6, 39) that cytokines inhibit parasitic and bacterial infections in a variety of cells by producing NO. Therefore, we then examined the relation between the anti-toxoplasma activities of cytokines and their ability to produce NO in HRPE cells. NO production by the cells was quantitated by measuring nitrite levels in the medium. After treatment with IL-1β, TNF-α, IFN-α, IFN-β, or IFN-γ for 24 h, the cultures were inoculated with *T. gondii*. After 5 days of incubation, supernatant fluids were assayed for the numbers of tachyzoites released and nitrite levels. These cytokine treatment of cultures did not augment the minimal levels of nitrite detected in medium-treated (control) cultures (0.42 ± 0.08 μM). IFN-γ at 100 U/ml completely inhibited *T. gondii* replication (Fig. 4) in the absence of NO production. In separate experiments, we determined nitrite levels in media from noninfected culture samples after 1, 2, and 3 days of cytokine treatment and did not detect NO production. In contrast, a combination of IFN-γ and IL-1β produced more than a 20-fold increase in NO production in HRPE cells (19.56 ± 3.50 μM), demonstrating that HRPE cells can produce NO under certain conditions (24).

In order to substantiate the apparent inhibition of *T. gondii* replication in the absence of NO, a second set of experiments were conducted with L-arginine (a substrate for NO production) and inhibitors of NO production. Addition of L-arginine or *N*ω-nitro-L-arginine and *N*ω-nitro-L-arginine methyl ester (inhibitors of NO synthase) to control and IFN-γ (10 or 100 U/ml)-treated cultures had no effect on *T. gondii* replication (data not shown). These results confirm that NO production is not involved in interferon-induced inhibition of *T. gondii* replication in HRPE cells.

Tryptophan reverses the effect of IFN-γ. Earlier studies have shown that treatment of cells with IFN-γ results in the depletion of the cellular pool of tryptophan by the induction of IDO, an enzyme which catalyzes the conversion of tryptophan to kynurenine. We then evaluated the ability of tryptophan to reverse the anti-toxoplasma activity of IFN-γ in HRPE cells. Cultures were treated with various combinations of IFN-γ and L-tryptophan, and replication of *T. gondii* was evaluated (Fig. 5). In the absence of tryptophan, IFN-γ exhibited a dose-dependent inhibition of *T. gondii* replication (Fig. 5). Tryptophan reversed the inhibition of *T. gondii* replication by IFN-γ. Moreover, the action of tryptophan was proportional to the concentration of tryptophan added to the cultures (Fig. 5 [20, 50, and 100 μg/ml]). For example, IFN-γ (20 U/ml) inhibited *T. gondii* replication by 80%, and this inhibition was reduced to 41% by tryptophan (100 μg/ml). The differences between 10 U of IFN-γ per ml without and with 100 μg of tryptophan per ml and 20 and 40 U of IFN-γ per ml without and with 50 and 100 μg of tryptophan per ml were highly significant (P, <0.01 [Student's t test]).

Induction of IDO by cytokines. IDO is an IFN-inducible enzyme that is linked to the depletion of an essential amino acid, L-tryptophan, by converting tryptophan to kynurenine. Since inhibition of *T. gondii* replication by IFN-γ is significantly reversed by the supplementation of culture medium with tryptophan, we evaluated expression of IDO in IFN-γ treated HRPE cultures. Total RNA prepared from untreated (control) and cytokine-treated HRPE cultures were reverse transcribed and amplified by PCR and analyzed by gel electrophoresis. A

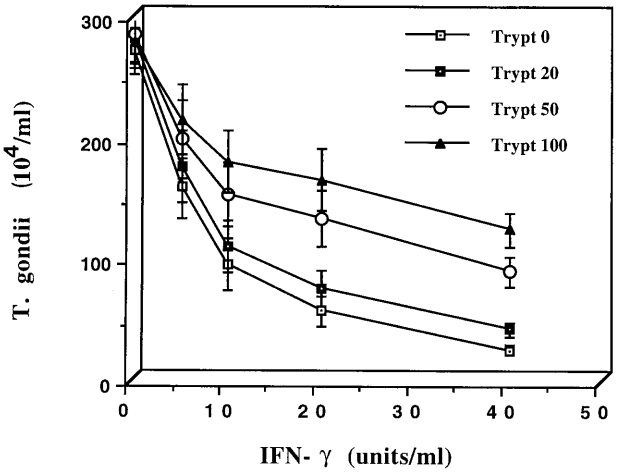

FIG. 5. L-Tryptophan blocks the ability of IFN-γ to inhibit *T. gondii* replication. HRPE cultures were incubated with various combinations of IFN-γ and L-tryptophan. After 24 h of incubation, the cultures were infected with tachyzoites of *T. gondii*, washed, and refed with medium containing the indicated concentrations of L-tryptophan. At days 1 to 4 postinoculation, concentrated tryptophan solutions were added to the cultures to maintain the original tryptophan concentration. Supernatant fluids were collected 5 days postinoculation to count the parasites released from the cells. Trypt 0, 20, 50, and 100, cultures that had L-tryptophan concentrations of 0, 20, 50, and 100 μg/ml, respectively. The results are means ± standard errors of the means for six experiments.

PCR product with an expected length of 662 bp was detected in these samples (data not shown). A PCR product of IFN-γ-treated HRPE cells was purified from the gel and subcloned into pCR II TA cloning vector. Sequencing of this cDNA revealed identity with the published nucleotide sequence of human IDO (8).

Northern blot analysis of the RNA isolated from cytokine-treated HRPE cultures was performed by using a subcloned IDO PCR product of 662 bp as a probe. An intense band of approximately 2.2 kb was observed in HRPE cells treated with IFN-γ for 24 h. In contrast, faint bands were seen in IFN-α-, IFN-β-, and TNF-α-treated cultures (Fig. 6A). In untreated cultures, the band was barely visible. Densitometric analysis of these blots yielded IDO-to-GAPDH ratios of 0.01, 0.20, 0.05, 0.35, and 2.30 for the control, and TNF-α-, IFN-α-, IFN-β-, and IFN-γ-treated samples, respectively. These results demon-

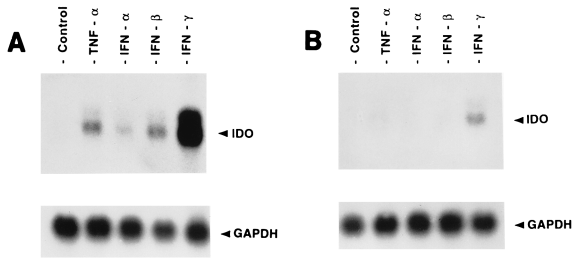

FIG. 6. Northern blot analysis of the expression of IDO mRNA in cytokine-treated HRPE cells. Two sets of cultures were treated with medium (control), TNF-α (10 ng/ml), IFN-α (1,000 U/ml), IFN-β (1,000 U/ml), or IFN-γ (100 U/ml). After 24 h of treatment, total RNA was prepared from one set of cultures for Northern blot analysis (A). In the other set of cultures, medium was removed, and the cells were washed twice to remove the cytokines and were incubated in cytokine-free medium. After 24 h, total RNA was prepared from these cultures for Northern blot analysis (B). Blots for GAPDH of the same samples are shown at the bottom of both panels. Arrowheads, positions of IDO and GAPDH mRNA.

strated that IFN-γ was the most potent inducer of IDO, after which came IFN-β, TNF-α, and IFN-α. Northern blotting prepared with RNA samples isolated from cultures 24 h after withdrawal of cytokine treatment is shown in Fig. 6B. In IFN-γ-treated cells, a less intense band was still noticeable, with an IDO-to-GAPDH ratio of 0.15. In contrast, bands were not seen in TNF-α-, IFN-α-, and IFN-β-treated cultures. Northern blots for GAPDH shown in Fig. 6A and B indicate that equal quantities of RNA were loaded in each of the lanes. At 48 h after the withdrawal of cytokine treatment, the level of IDO mRNA returned to the level of untreated cultures, at which no bands are visible (data not shown).

DISCUSSION

Retinochoroiditis, which is caused by congenital or acquired ocular toxoplasmosis, involves inflammation and necrosis of the neuroretina, RPE, and choroid (18, 34, 38, 47, 52). The presence of cysts and free forms of *T. gondii*, a protozoan parasite responsible for retinochoroiditis, was demonstrated in both the neuroretina and the RPE (16, 17, 33, 52). However, the nature of *T. gondii* replication within the retina and the role of cytokines in controlling intracellular multiplication of the parasite have not been investigated. Therefore, we studied the replication of *T. gondii* in HRPE cultures and evaluated the mechanisms by which interferons inhibit the growth of this parasite. *T. gondii* replicated readily in HRPE, occasionally forming intracellular cyst-like structures (Fig. 1D, E, and F). Typically, after multiplication inside the cell, parasites were released into the supernatant fluid and invaded neighboring cells, forming a focal area of infection (Fig. 1A, B, and C). The presence of tachyzoites within the cytoplasm of the cells and in the plaques was confirmed by immunostaining with parasite-specific antibodies. We quantitated replication of *T. gondii* in HRPE by counting the numbers of plaques formed in the cultures and/or counting parasites released into the supernatant fluids. In this study, we report that interferons inhibit replication of *T. gondii* in HRPE. This inhibition was not associated with NO production. Inhibition of *T. gondii* replication was associated with L-tryptophan starvation as a result of the induction of IDO, an enzyme that converts tryptophan to *N*-formylkynurenine.

In human ocular toxoplasmosis patients, the levels of interferons and cytokines such as TNF-α, IL-1, and IL-6 in the ocular microenvironment are not known. However, increased levels of mRNA for IFN-γ and TNF-α were demonstrated by reverse transcription-PCR analysis in *T. gondii*-infected mouse eyes (17). In the present study, we investigated the effects of interferons and cytokines to delineate their role in the control of *T. gondii* replication in a pathogenesis-relevant primary human retinal cell, the RPE cell. IFN-γ was found to be the most potent anti-toxoplasma agent in HRPE cells, which was followed by IFN-β, IFN-α, and TNF-α. Inhibition of *T. gondii* replication in HRPE cells by IFN-γ was reversed by anti-IFN-γ MAb. Previous studies have indicated the potent inhibitory effects of recombinant IFN-γ on *T. gondii* replication in human macrophages (31), in a human fibroblast cell line (42), and in human monocyte-derived macrophages obtained from adults and newborns (53). Recently, ovine recombinant IFN-γ was shown to suppress the growth of *T. gondii* in alveolar macrophages and an ovine fibroblast cell line (36). In HRPE cells, we found that recombinant IFN-α and IFN-β also exhibited significant inhibitory effect on *T. gondii* replication, even though to a lesser extent than recombinant IFN-γ (Fig. 4). Such inhibitory actions of IFN-α and IFN-β on *T. gondii* replication were seen in human monocyte-derived macrophages (44, 53). In contrast, recombinant IFN-α and IFN-β were reported to have no effect on *T. gondii* replication in a human fibroblast cell line (42) and in human monocyte-derived macrophages (28, 32). These observed differences in IFN-α and IFN-β antiparasitic actions by various investigators could be due to the inherent properties of the types of cells used in each study.

The roles of various cytokines in the defense against *T. gondii* infection and in the interaction between mammalian cells and the parasite have been recently reviewed (3, 46). The role of IFN-γ in the resistance to *T. gondii* infection in vivo in mice was demonstrated on the basis of the protection offered by the exogenous administration of IFN-γ as well as susceptibility to infection in mice by the administration of anti-IFN-γ MAb (26, 49, 50). Protective actions of IFN-β in vivo in BALB/c mice (35) and of TNF-α in Swiss Webster mice (5, 23) infected with *T. gondii* have also been reported.

Interferons act as antimicrobial agents by activating macrophages, lymphocytes, and other cells (i) by producing oxygen-reactive metabolites, (ii) by producing NO, (iii) by inducing a double-stranded-RNA-dependent protein kinase that phosphorylates and inactivates the elongation factor eIF2, and (iv) by inducing oligonucleotide 2,5′-adenylate synthetase that synthesizes polyadenylates which activate endonucleases that degrade RNA (2, 14, 27). Interferons also induce IDO, which converts L-tryptophan, an essential amino acid, to *N*-formylkynurenine, thereby depleting this essential amino acid and subjecting parasites to tryptophan starvation (40, 41, 43). The anti-toxoplasma activities of interferons have been mostly attributed by various investigators to NO and/or tryptophan starvation by the induction of IDO. Murine macrophages (1) and microglia (6) activated by IFN-γ and LPS produced NO and inhibited the growth of *T. gondii*. Human astrocytes activated by IFN-γ and IL-1β also produced NO and inhibited *T. gondii* replication (39). The participation of NO in the inhibition of *T. gondii* growth in these studies was supported by the reversal of antitoxoplasmic activity in the presence of the inhibitors of NO synthase, an enzyme responsible for NO production. Since the effects of IFN-γ alone were not evaluated in these studies, it is not possible to determine whether IFN-γ by itself could have inhibited *T. gondii* growth in the absence of NO production. In HRPE cells, IFN-γ alone at a 100-U/ml concentration completely inhibited *T. gondii* replication, while TNF-α, IFN-α, and IFN-β exhibited significant inhibition of *T. gondii* replication at concentrations that did not induce NO production. We have recently shown that HRPE cells were capable of producing NO only in the presence of a combination of IFN-γ and IL-1β but not when only one of the cytokines was present (24). Our studies clearly demonstrate that interferons and TNF-α inhibit *T. gondii* replication in HRPE cells by NO-independent mechanisms, although we cannot exclude the role of NO in antitoxoplasmic activity in HRPE.

We then examined the induction of IDO by Northern blot analysis to evaluate the mechanisms of antiparasitic action by interferons. Induction of IDO mRNA in IFN-γ-treated HRPE cultures was more than 200-fold higher than that in control cultures. In TNF-α-, IFN-α-, and IFN-β-treated cultures, induction of IDO mRNA increased by a range of 5- to 35-fold (Fig. 6). In HRPE cells, IFN-γ, the most potent inducer of IDO mRNA, was found to be the most potent inhibitor of *T. gondii* replication. Significant induction of IDO activity in the human fibroblast cell line after 24 to 48 h of treatment with IFN-γ was reported by Pfefferkorn et al. (43). In human monocyte-derived macrophages, IFN-α, IFN-β, and IFN-γ were found to induce IDO activity after 72 h of treatment (53). IFN-γ was twice as potent as IFN-α or IFN-β in IDO induction

in these cells. In human fibroblast cells, expression of a 2.2-kb IDO mRNA was induced 100-fold by IFN-γ, whereas only 10-fold induction was observed in IFN-α- and IFN-β-treated cells (4). The role of IDO induction and consequent depletion of cellular tryptophan levels as the major cause of the antitoxoplasma activity of IFN-γ arose from transfection studies (9, 19). These investigators used human fibroblast cell lines that were transfected with IDO cDNA cloned into the expression vector and found that the inhibition of *T. gondii* growth was proportional to the IDO activities of these cell lines.

In HRPE cells, an epithelial cell line with macrophage-like properties (12), IFN-γ, IFN-β, IFN-α, and TNF-α induced expression of IDO mRNA. Inhibition of *T. gondii* growth in HRPE cells by these agents was closely correlated with their ability to induce IDO (Fig. 4 and 6). Moreover, the role of IFN-γ-induced IDO in the inhibition of *T. gondii* in HRPE was confirmed by the experiments which showed reversal of IFN-γ actions by the addition of various concentrations of L-tryptophan to the medium (Fig. 5). Since inhibition by IFN-γ was not completely reversed by the addition of tryptophan, it is possible that additional mechanisms operate in the protection offered by interferons. Studies of reverse transcription-PCR analysis of oligoadenylate synthetase expression have indicated that IFN-α and IFN-β induce this enzyme to a level much higher than that of IFN-γ (unpublished observations). Therefore, induction of IDO and depletion of cellular L-tryptophan appeared to be the major mechanisms by which interferons inhibit *T. gondii* growth in HRPE, which are similar to those proposed by Pfefferkorn et al. for the human fibroblast cell line (40, 41).

The RPE acts as a semipermeable barrier between the neuroretina and choroid and is shown to play an important role in infectious, inflammatory, and degenerative diseases (54). In some parasitic and viral infections and in other diseases, disorganization and destruction of neuroretinal and choroidal elements are accompanied by necrosis and degeneration of the RPE cell (54). In retinochoroiditis induced by *T. gondii* in humans, the presence of free and cyst forms of this parasite in the RPE, and the neuroretina was demonstrated (16, 33, 52). In the present report, we have demonstrated that *T. gondii*-infected HRPE cells are a useful in vitro model to investigate *T. gondii* replication and cytokine-induced modulation of parasite replication in a pathogenesis-relevant retinal cell.

REFERENCES

1. **Adams, L. B., J. B. Hibbs, R. D. Tainter, and J. L. Krahenbuhl.** 1990. Microbiostatic effect of murine-activated macrophages for *Toxoplasma gondii*. J. Immunol. **144:**2725–2729.
2. **Arai, K., F. Lee, A. Miyajima, S. Miyatake, N. Arai, and T. Yokota.** 1990. Cytokines: coordinators of immune and inflammatory responses. Annu. Rev. Biochem. **59:**783–836.
3. **Beaman, M. H., S.-Y. Wong, and J. S. Remington.** 1992. Cytokines, toxoplasma and intracellular parasitism. Immun. Rev. **127:**97–117.
4. **Caplen, H. S., and S. L. Gupta.** 1988. Differential regulation of a cellular gene by human interferon-γ and interferon-α. J. Biol. Chem. **263:**332–339.
5. **Chang, H. R., G. E. Grau, and J.-C. Pechere.** 1990. Role of TNF and IL-1 in infections with *Toxoplasma gondii*. Immunology **69:**33–37.
6. **Chao, C. C., W. R. Anderson, S. Hu, G. Gekker, A. Martella, and P. K. Peterson.** 1993. Activated microglia inhibit multiplication of *Toxoplasma gondii* via a nitric oxide mechanism. Clin. Immunol. Immunopathol. **67:**178–183.
7. **Chao, C. C., G. Gekker, S. Hu, and P. K. Peterson.** 1994. Human microglial cell defense against *Toxoplasma gondii*. The role of cytokines. J. Immunol. **152:**1246–1252.
8. **Dai, W., and S. L. Gupta.** 1990. Molecular cloning, sequencing and expression of human interferon-γ-inducible indoleamine 2,3-dioxygenase cDNA. Biochem. Biophys. Res. Commun. **168:**1–8.
9. **Dai, W., H. Pan, O. Kwok, and J. P. Dubey.** 1994. Human indoleamine 2,3-dioxygenase inhibits *Toxoplasma gondii* growth in fibroblast cells. J. Interferon Res. **14:**313–317.
10. **Detrick, B. D., D. Newsome, C. Percopo, and J. J. Hooks.** 1985. Class II antigen expression and gamma interferon modulation of monocytes and retinal pigment epithelial cells from patients with retinitis pigmentosa. Clin. Immunol. Immunopathol. **36:**201–211.
11. **Dubey, J. P., and C. P. Beattie.** 1988. Toxoplasmosis of animals and man. CRC Press Inc., Boca Raton, Fla.
12. **Elner, V. M., T. Schaffner, K. Taylor, and S. Glagov.** 1981. Immunophagocytic properties of retinal pigment epithelium cells. Science **211:**74–76.
13. **Elner, V. M., R. M. Strieter, S. G. Elner, M. Baggiolini, I. Lindley, and S. L. Kunkel.** 1990. Neutrophil chemotactic factor (IL-8) gene expression by cytokine-treated retinal pigment epithelial cells. Am. J. Pathol. **136:**745–750.
14. **Farrar, M. A., and R. D. Schreiber.** 1993. The molecular cell biology of interferon-γ and its receptor. Annu. Rev. Immunol. **11:**571–611.
15. **Frenkel, J. K.** 1988. Pathophysiology of toxoplasmosis. Parasitol. Today **4:**273–278.
16. **Friedman, A. H.** 1993. Uveitis affecting the retina and posterior segment, p. 37–70. *In* W. R. Freeman (ed.), Practical atlas of retinal diseases and therapy. Raven Press, New York.
17. **Gazzinelli, R. T., A. Brezin, Q. Li, R. B. Nussenblatt, and C. C. Chan.** 1994. *Toxoplasma gondii*: acquired ocular toxoplasmosis in the murine model, protective role of TNF-α and IFN-γ. Exp. Parasitol. **78:**217–229.
18. **Glasner, P. D., C. Silveira, D. Kruszon-Moran, et. al.** 1992. An unusually high prevalence of ocular toxoplasmosis in southern Brazil. Am. J. Ophthalmol. **114:**136–144.
19. **Gupta, S. L., J. M. Carlin, P. Pyati, W. Dai, E. R. Pfefferkorn, and M. J. Murphy, Jr.** 1994. Antiproliferative effects of indoleamine 2,3-dioxygenase enzyme expression in human fibroblasts. Infect. Immun. **62:**2277–2284.
20. **Henderly, D. E., A. J. Genstler, R. E. Smith, and N. A. Rao.** 1987. Changing patterns of uveitis. Am. J. Ophthalmol. **103:**131–136.
21. **Holland, G. N., R. E. Engstrom, Jr., B. J. Glasgow, et. al.** 1988. Ocular toxoplasmosis in patients with the acquired immunodeficiency syndrome. Am. J. Ophthal. **106:**653–657.
22. **Hughes, H. P. A., L. Hudson, and D. G. Fleck.** 1986. In vitro culture of *Toxoplasma gondii* in primary and established cell lines. Int. J. Parasitol. **16:**317–322.
23. **Johnson, L. L.** 1992. A protective role for endogenous tumor necrosis factor in *Toxoplasma gondii* infection. Infect. Immun. **60:**1979–1983.
24. **Kutty, R. K., G. Kutty, J. J. Hooks, B. Wiggert, and C. N. Nagineni.** 1995. Transforming growth factor-β inhibits the cytokine-mediated expression of the inducible nitric oxide synthase mRNA in human retinal pigment epithelial cells. Biochem. Biophys. Res. Commun. **215:**386–393.
25. **Lindsay, D. S., J. P. Dubey, B. L. Blagburn, and M. Toivio-Kinnucan.** 1991. Examination of tissue cyst formation by *Toxoplasma gondii* in cell cultures using bradyzoites, tachyzoites, and sporozoites. J. Parasitol. **77:**126–132.
26. **McCabe, R. E., B. J. Luft, and J. S. Remington.** 1984. Effect of murine interferon gamma on murine toxoplasmosis. J. Infect. Dis. **150:**961–962.
27. **McNair, A. N. B., and I. M. Kerr.** 1992. Viral inhibition of the interferon system. Pharmacol. Ther. **56:**79–95.
28. **Murray, H. W., A. Szuro-Sudol, D. Wellner, M. J. Oca, A. M. Granger, D. M. Libby, C. D. Rothermel, and B. Y. Rubin.** 1989. Role of tryptophan degradation in respiratory burst-independent antimicrobial activity of gamma interferon-stimulated human macrophages. Infect. Immun. **57:**845–849.
29. **Nagineni, C. N., B. Detrick, and J. J. Hooks.** 1994. Synergistic effects of gamma interferon on inflammatory mediators that induce interleukin-6 gene expression and secretion by human retinal pigment epithelial cells. Clin. Diagn. Lab. Immunol. **1:**569–577.
30. **Nagineni, C. N., R. K. Kutty, B. Detrick, and J. J. Hooks.** Inflammatory cytokines induce intercellular adhesion molecule-1 (ICAM-1) mRNA synthesis and protein secretion by human retinal pigment epithelial cell cultures. Cytokine, in press.
31. **Nathan, C. F., H. W. Murray, M. E. Wiebe, and B. Y. Rubin.** 1983. Identification of interferon-γ as the lymphokine that activates human macrophages: oxidative metabolism and antimicrobial activity. J. Exp. Med. **158:**670–689.
32. **Nathan, C. F., T. J. Prendergast, M. E. Wiebe, E. R. Stanley, E. Platzer, H. G. Remold, K. Welte, B. Y. Rubin, and H. W. Murray.** 1984. Activation of human macrophages. Comparison of other cytokines with interferon-γ. J. Exp. Med. **160:**600–605.
33. **Nicholson, D. H., and E. B. Wolchok.** 1976. Ocular toxoplasmosis in an adult receiving long-term corticosteroid therapy. Arch. Ophthalmol. **94:**248–254.
34. **Nussenblatt, R. B., S. M. Whitcup, and A. G. Palestine.** 1996. Ocular toxoplasmosis, p. 211–228. *In* R. B. Nussenblatt, S. M. Whitcup, and A. G. Palestine (ed.), Uveitis. Fundamentals and clinical practice. Mosby-Year Book, St. Louis. Mo.
35. **Orellana, M. A., Y. Suzuki, F. Araujo, and J. S. Remington.** 1991. Role of beta interferon in resistance to *Toxoplasma gondii* infection. Infect. Immun. **59:**3287–3290.
36. **Oura, C. A. L., E. A. Innes, J. M. Wastling, G. Entrican, and W. R. M. Panton.** 1993. The inhibitory effect of ovine recombinant interferon-gamma on intracellular replication of *Toxoplasma gondii*. Parasite Immunol. **15:**535–538.
37. **Percopo, C., J. J. Hooks, T. Shinohara, R. Caspi, and B. Detrick.** 1990.

Cytokine mediated activation of a neuronal retinal resident cells provokes antigen presentation. J. Immunol. **145:**4101–4107.
38. **Perkins, E. S.** 1973. Ocular toxoplasmosis. Br. J. Ophthalmol. **57:**1–17.
39. **Peterson, P. K., G. Gekker, S. Hu, and C. C. Chao.** 1995. Human astrocytes inhibit intracellular multiplication of *Toxoplasma gondii* by nitric oxide-mediated mechanism. J. Infect. Dis. **171:**516–518.
40. **Pfefferkorn, E. R.** 1984. Interferon-γ blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. Proc. Natl. Acad. Sci. USA **81:**908–912.
41. **Pfefferkorn, E. R., M. Eckel, and S. Rebhun.** 1986. Interferon-γ suppresses the growth of *Toxoplasma gondii* in human fibroblasts through starvation for tryptophan. Mol. Biochem. Parasitol. **20:**215–224.
42. **Pfefferkorn, E. R., and P. M. Guyre.** 1984. Inhibition of growth of *Toxoplasma gondii* in cultured fibroblasts by human recombinant gamma interferon. Infect. Immun. **44:**211–216.
43. **Pfefferkorn, E. R., S. Rebhun, and M. Eckel.** 1986. Characterization of an indoleamine 2,3-dioxygenase induced by gamma-interferon in cultured human fibroblasts. J. Interferon Res. **6:**267–279.
44. **Schmitz, J. L., J. M. Carlin, E. C. Borden, and G. I. Byrne.** 1989. Beta interferon inhibits *Toxoplasma gondii* growth in human monocyte-derived macrophages. Infect. Immun. **57:**3254–3256.
45. **Shirihata, T., A. Mori, H. Ishikawa, and H. Goto.** 1986. Strain differences of interferon generating capacity and resistance in toxoplasma infected mice. Microbiol. Immunol. **30:**1307–1316.
46. **Sibley, L. D.** 1993. Interactions between *Toxoplasma gondii* and its mammalian host cells. Semin. Cell Biol. **4:**335–344.
47. **Silveira, C., R. Belfort, Jr., M. Burnier, Jr., and R. Nussenblatt.** 1988. Acquired toxoplasmic infection as the cause of toxoplasmic retinochoroiditis in families. Am. J. Ophthalmol. **106:**362–364.
48. **Slavin, M. A., J. D. Meyers, J. S. Remington, and R. C. Hackman.** 1994. *Toxoplasma gondii* infection in marrow transplant recipients: a 20 year experience. Bone Marrow Transplant. **13:**549–557.
49. **Suzuki, Y., F. K. Conley, and J. S. Remington.** 1989. Importance of endogenous IFN-γ for prevention of toxoplasmic encephalitis in mice. J. Immunol. **143:**2045–2050.
50. **Suzuki, Y., N. A. Orellana, R. D. Schreiber, and J. S. Remington.** 1988. Interferon-γ: the major mediator of resistance against *Toxoplasma gondii*. Science **240:**516–518.
51. **Tabbara, K. F.** 1990. Disruption of the choroidoretinal interface by Toxoplasma. Eye **4:**366–373.
52. **Wilder, H. P.** 1952. Toxoplasma chorioretinitis in adults. Arch. Ophthalmol. **48:**127–136.
53. **Wilson, C. B., and J. Westall.** 1985. Activation of neonatal and adult human macrophages by alpha, beta, and gamma interferons. Infect. Immun. **49:**351–356.
54. **Zinn, K. M., and M. F. Marmor.** 1979. The retinal pigment epithelium. Harvard University Press, Cambridge, Mass.

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