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Activation of Neonatal and Adult Human Macrophages by Alpha, Beta, and Gamma Interferons

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Increasing evidence suggests that gamma interferon (IFN-γ) is the major or sole factor in human lymphokines which activates blood monocyte-derived macrophages (Mϕ) to inhibit or kill *Toxoplasma gondii* and certain other intracellular pathogens. In the current studies, we found that IFN-γ effectively activated tissue Mϕ from adults (peritoneal Mϕ) and from newborns (placental Mϕ) as well as blood-derived Mϕ from adults and from newborns to kill or to inhibit the replication of *T. gondii*. Results with purified and recombinant IFN-γ and with adult and newborn Mϕ were similar. IFN-γ-treated Mϕ were equally or more active against *T. gondii* than were freshly isolated monocytes and Mϕ. Recombinant IFN-αA and IFN-β were less effective than IFN-γ. IFN-γ also inhibited survival and replication of *T. gondii* in WISH cells more effectively than did IFN-α and IFN-β. These findings are consistent with an important role for IFN-γ in the control of *Toxoplasma* infection and indicate that the anti-*Toxoplasma* activity of resting and IFN-γ-activated adult and neonatal Mϕ is similar. The increased susceptibility of neonates to *T. gondii* is not due to a defect in Mϕ effector function.

Recent studies by ourselves (24) and others (6, 10, 12–14, 19, 20, 22, 29) have implicated gamma interferon (IFN-γ) as the major macrophage-activating factor in preparations of crude lymphokines. Nathan et al. (14) found that recombinant IFN-γ (rIFN-γ) activated adult monocyte-derived macrophages (Mϕ) to kill or inhibit the replication of *Toxoplasma gondii*, whereas IFN-α, IFN-β, and lymphokines (LK) other than IFN-γ did not. Whether IFN-γ can activate human tissue Mϕ against *T. gondii* has not been determined. The current studies compare the effects of different preparations of IFN-γ, rIFN-αA, and rIFN-β on the survival and replication of *T. gondii* in tissue Mϕ and blood monocyte-derived Mϕ from human adults and neonates. The effects of these preparations on the survival and replication of *T. gondii* in WISH cells, a cell line of fetal amnion origin, was also examined.

MATERIALS AND METHODS

Reagents. Reagents were obtained from Sigma Chemical Co., St. Louis, Mo., unless otherwise indicated. Hanks balanced salt solution, calf serum, L-glutamine, penicillin, and streptomycin were obtained from GIBCO Laboratories, Grand Island, N.Y. RPMI 1640 containing 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer was obtained from MA Bioproducts, Walkersville, Md. When analyzed by the *Limulus* amebocyte lysate assay (Pyrotell Associates of Cape Cod, Inc., Woods Hole, Mass.), the RPMI 1640 contained <0.06 ng of reference *Escherichia coli* endotoxin per ml. However, in some assays, RPMI 1640 containing 0.1 to 1.0 ng of endotoxin per ml was used with similar results. Fetal calf serum was obtained from Sterile Systems, Inc., Logan, Utah; it contained 0.07 to 0.13 ng of endotoxin per ml and lacked detectable *Toxoplasma* antibody (<1:4) in the Sabin Feldman dye test. Concanavalin A, Ficoll-Paque and Percoll were obtained from Pharmacia Fine Chemicals, Piscataway, N.J. [^{3}H]thymidine (6.7 Ci/mmol), [^{3}H]uracil (40 mCi/mmol), Omnifluor, and Aquasol 2 were obtained from New England Nuclear Corp., Boston, Mass. Glutaraldehyde was obtained from Ted Pella, Inc., Irvine, Calif. Diff Quik was obtained from Dade Diagnostics, Aguado, P.R. Partially purified human IFN-γ, prepared by stimulating buffy coat cells with staphylococcal enterotoxin A and purified by column chromatography, was obtained from the National Institutes of Health (Gg 23-901-530) or from B. Y. Rubin, Sloan Kettering Cancer Institute, Rye, N.Y.; these preparations contained ~0.1 ng of reference endotoxin per 1,000 U of activity. Two preparations of rIFN-γ from *E. coli* were provided by Genentech, Inc., San Francisco, Calif.; these preparations lacked detectable endotoxin (<0.06 ng/ml). The standard preparation of rIFN-γ is that originally described by Gray et al. (3); the second preparation was modified by deleting Cys-Tyr-Cys and adding methionine at the amino-terminal end of the polypeptide (18). Unless otherwise indicated, the studies described were done with the standard preparation of rIFN-γ. rIFN-αA and rIFN-β were obtained from P. Trown, Hoffmann-LaRoche Inc., Nutley, N.J. A human IFN-α standard (G-023-902-527) was obtained from the National Institutes of Health.

Cell preparations. Blood mononuclear cells were obtained by Ficoll-Paque density gradient separation of adult peripheral blood and newborn cord blood as previously described (24, 26). Monolayers of adherent monocytes were prepared and cultured on 15-mm glass cover slips in 16-mm wells of Linbro trays (Linbro Chemical Co., Hamden, Conn.) for the indicated times in RPMI 1640 containing 2 mM L-glutamine, 25 mM HEPES buffer, 50 U of penicillin G per ml, 50 μg of streptomycin (RPMI 1640) per ml, and 20% autologous or human type AB serum (RPMI 1640 plus AS) that lacked detectable *Toxoplasma* antibodies. Mϕ were obtained from human term placentas by digestion of washed, minced tissue

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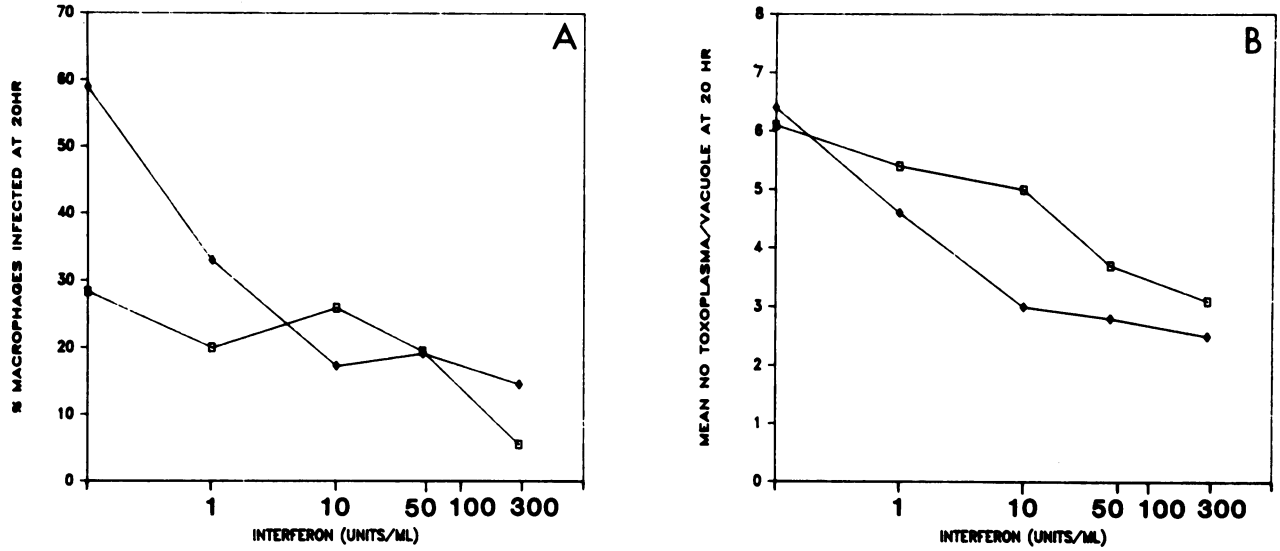

FIG. 1. Effects of rIFN-γ on survival (A) and replication (B) of *T. gondii* in adult monocyte-derived Mϕ (□) and in placental tissue Mϕ (◇). Cells were cultured in vitro for 4 to 5 days, rIFN-γ was added in the indicated concentrations for 72 h, and then Mϕ were infected with *T. gondii*. Monolayers were then processed and examined microscopically as described in the text. Results are the mean of 4 to 17 experiments with adult Mϕ and 3 to 12 experiments with placental Mϕ. Immediately after the 1-h infection period, the percentage of infected adult control Mϕ and interferon-treated Mϕ (300 U/ml) was 37.6 ± 12.5 and 38.4 ± 13.7, respectively, and that of infected placental control Mϕ and interferon-treated Mϕ was 65.6 ± 18.0 and 38.4 ± 24.8, respectively. The mean number of parasites per vacuole was 1.0 to 1.2 for each.

with collagenase and DNase, sequential density gradient separation on Ficoll-Plaque and Percoll, and differential adherence as previously described (25); these cells were cultured in RPMI 1640 plus 10% fetal calf serum and are principally fetal in origin. Peritoneal effluent was obtained from women undergoing diagnostic laparoscopy for infertility or for endometriosis and from a patient receiving chronic intermittent peritoneal dialysis at times when she was free of infection. Peritoneal cells were collected by centrifugation at 200 × *g* for 10 min, suspended in Hanks balanced salt solution, and centrifuged on Ficoll-Plaque at 700 × *g* for 15 min. The cells recovered at the interface were washed twice in Hanks balanced salt solution by centrifugation at 200 × *g* for 10 min and suspended in RPMI 1640 plus AB serum. Cells were enumerated in a hemacytometer, and the percentages of Mϕ and granulocytes were determined on smears stained for nonspecific esterase (7) and for myeloperoxidase (5). Peritoneal cell suspensions contained 58.4 ± 19.2% Mϕ and 10.2 ± 20.6% granulocytes, and the remainder consisted of lymphocytes. Monolayers of peritoneal Mϕ were prepared as previously described for blood monocyte monolayers. Monolayers of each cell type were routinely >95% Mϕ or monocytes.

Preparation of LK. Adult blood mononuclear cells were resuspended in RPMI 1640 plus 5% AB serum at a concentration of 5 × 10^6 cells per ml. Cells were cultured in the presence of an optimal concentration of concanavalin A (40 to 50 μg/ml) for 24 h at 37°C; culture supernatants were collected, adsorbed with Sephadex G10 to remove concanavalin A, and filter sterilized as previously described (24).

Interaction of *T. gondii* with Mϕ. Monolayers of monocytes and Mϕ were either used within 2 h of preparation or were cultured in vitro for the intervals indicated before use (see Table 1). Fresh peritoneal or placental Mϕ were used either 2 or 18 h after isolation; results with 2- and 18-h-cultured cells were similar, so they were pooled. Where indicated, culture supernatants from ConA-stimulated adult blood mononuclear cells (LK) in a final concentration of 30% or IFN preparations in the indicated concentrations were added to monolayers for the indicated intervals before use.

Monolayers of monocytes and Mϕ were infected with *T. gondii*, and survival and replication were assessed microscopically or by uptake of [^{3}H]uracil as previously described (8, 9, 27, 28).

IFN assays. IFN activity was determined by vesicular stomatitis virus plaque reduction as previously described (11, 24), except that WISH cells rather than fibroblasts were used. Standard preparations of IFN-γ were used to determine the IFN content in the rIFN-γ and in adult LK; standard preparations of IFN-α were used to determine the IFN content in the rIFN-αA and rIFN-β. Under our assay condition, the IFN contents of the standard and modified rIFN-γ were 4.8 × 10^7 U/ml and 6.7 × 10^7 U/ml, whereas the supplier indicated their activity to be 1.3 × 10^7 U/ml and 3.7 × 10^7 U/ml, respectively. Similarly, under our assay conditions the rIFN-αA and the rIFN-β contained 3.9 × 10^5 U/ml and 6.7 × 10^5 U/ml, whereas the supplier indicated their activity to be 6 × 10^5 U/ml and 1 × 10^6 U/ml, respectively. The activity indicated in the text is that determined by the supplier.

Statistics. Results are presented as mean ± standard deviation (SD). The significance of the differences between means was evaluated by a two-tailed Student's *t* test or by a paired *t* test as appropriate (21).

RESULTS

Concentration-dependent effects of rIFN-γ on intracellular *T. gondii*. Survival and replication of *T. gondii* in adult monocyte-derived Mϕ and placental Mϕ were inhibited by treatment of these cells with rIFN-γ in a concentration-dependent manner (Fig. 1). Maximum effects occurred at 50 to 300 U/ml; greater concentrations of IFN-γ were not more effective. As previously described (24), unpurified LK,

TABLE 1. Effects of in vitro culture, LK, and rIFN-γ on survival and replication of *T. gondii* in freshly isolated and cultured monocytes[a]

Cell prepn	Adult monocytes			Cord monocytes		
	% Infected at 1 h[b]	20 h		% Infected at 1 h	20 h	
		% Infected	$\bar{X}$/vacuole (no. of expt)		% Infected	$\bar{X}$/vacuole (no. of expt)
Cultured for 1 h (fresh)	23.3 ± 1.0	12.5 ± 2.9	2.6 ± 0.3 (4)	11.3 ± 2.6	9.3 ± 6.0	2.5 ± 0.2 (4)
Cultured for 4 to 5 days						
Control	27.5 ± 12.6	15.8 ± 8.7	4.2 ± 0.7 (5)	35.3 ± 11.0	16.7 ± 9.1	4.8 ± 1.8 (3)
+ LK	6.0 ± 0.0	4.8 ± 4.2	2.6 ± 1.2[c] (4)	15.0	13.5 ± 0.7	4.0 ± 0.4 (2)
+ rIFN-γ	38.3 ± 19.4	4.8 ± 4.4	2.5 ± 1.0[c] (5)	24.5 ± 0.7	9.0 ± 6.1	1.9 ± 0.8[d] (3)
Cultured for 7 to 8 days						
Control	37.6 ± 12.5	31.4 ± 19.1	5.8 ± 0.9 (18)	45.3 ± 12.2	33.3 ± 10.2	5.8 ± 0.7 (4)
+ LK	25.8 ± 7.5	10.2 ± 12.7[c]	3.5 ± 1.2[e] (10)	53.0 ± 4.2	14.5 ± 7.9[c]	3.1 ± 0.9[e] (4)
+ rIFN-γ	38.3 ± 13.5	5.6 ± 3.7[e]	2.7 ± 1.1[e] (17)	31.0 ± 11.3	9.6 ± 8.4[c]	2.1 ± 1.2[e] (4)

[a] Cells were cultured in vitro for 1 h (fresh monocytes) or for the indicated time. Where indicated, 30% LK or 300 U of rIFN-γ per ml was added 72 h before the assay. Cells were infected with *T. gondii* for 1 h, and the percentage of infected cells and mean number of *T. gondii* organisms per infected cell or mean number of *T. gondii* organisms per vacuole were determined immediately (1 h) or at 20 h. Results are means ± standard deviations.

[b] The mean number of *T. gondii* per infected cell at 1 h was similar for adult and cord cells and ranged from 1.1 to 1.4 for fresh cells, 1.3 to 2.5 for 4- to 5-day-cultured cells, and 1.5 to 2.1 for 7- to 8-day-cultured cells, respectively. The mean number of parasites per vacuole at 1 h was 1.0 to 1.2 for each.

[c] $P < 0.05$ compared to controls.

[d] $P < 0.06$ compared to controls.

[e] $P < 0.01$ compared to controls.

which contained ~900 U of IFN per ml, had similar effects at the optimal 30% final concentration (≈270 U/ml). Exposure of Mϕ to 300 U of rIFN-γ per ml for as little as 4 h had an inhibitory effect on *Toxoplasma* replication, whereas 24 h was necessary to inhibit survival of *T. gondii* (data not shown). Maximum inhibition of both occurred after 48 to 72 h of exposure to rIFN-γ.

Effects of in vitro culture, LK, and rIFN-γ on the interaction of monocytes and Mϕ with *T. gondii*. When blood monocytes from adults and newborns were cultured in vitro before they were infected, they became progressively less able to kill or inhibit the replication of *T. gondii* (Table 1). Fresh peritoneal and placental Mϕ were more permissive than fresh monocytes; both became still more permissive after they were cultured in vitro (Table 2).

Exposure to IFN-γ enhanced the ability of each type of Mϕ to kill or inhibit the replication of *T. gondii* (Tables 1 and 2). Of note, Mϕ that were treated with rIFN-γ or LK for the final 72 h of a culture period of 4 to 5 or 7 to 8 days were similar to fresh monocytes in their ability to kill or inhibit the replication of *T. gondii*. Recombinant IFN-γ was equally or more effective than LK.

We also determined the effects of rIFN-γ on survival and replication of *T. gondii* in Mϕ by the uptake of [^{3}H]uracil. [^{3}H]uracil uptake by control Mϕ and by Mϕ treated with 300 U of rIFN-γ per ml for 72 h was 89,773 ± 2,318 cpm and

TABLE 2. Effects of in vitro culture, LK, and rIFN-γ on survival and replication of *T. gondii* in adult peritoneal Mϕ and neonatal placental Mϕ[a]

Cell prepn	Peritoneal Mϕ			Placental Mϕ		
	% Infected at 1 h[b]	20 h		% Infected at 1 h	20 h	
		% Infected	$\bar{X}$/vacuole (no. of expt)		% Infected	$\bar{X}$/vacuole (no. of expt)
Cultured for 1 h (fresh)	31.0 ± 20.5[c]	25.8 ± 23.7	5.0 ± 0.9 (4)	32.0 ± 19.1	44.3 ± 39.2	3.3 ± 0.6 (3)
Cultured for 4 to 5 days						
Control	42.0 ± 24.3	22.0 ± 4.6	6.4 ± 0.3 (3)	64.3 ± 21.6	61.7 ± 31.1	5.9 ± 0.6 (3)
+ LK	18.0	7	2.6 (1)	48.0	10.8 ± 1.8	2.6 ± 0.1[d] (2)
+ rIFN-γ	19.0 ± 0.0	2.0 ± 1.4[d]	3.2 ± 0.3[d] (3)	48.9 ± 15.4	23.0 ± 11.3	2.2 ± 0.4[d] (3)
Cultured for 7 to 8 days						
Control	67.0 ± 7.1	70.0 ± 4.2	6.3 ± 0.4 (2)	65.6 ± 18.0	58.8 ± 25.6	6.2 ± 0.7 (11)
+ LK	ND	ND	ND	66.0	25.0 ± 26.1	2.9 ± 1.2[d] (4)
+ rIFN-γ	76	4.5 ± 2.1[d]	1.9 ± 0.1[e] (2)	42.8 ± 18.1[e]	13.3 ± 8.7[d]	2.2 ± 0.7[d] (12)

[a] Procedures were as indicated in Table 1, footnote *a*, except that fresh cells were cultured for 2 to 18 h before use.

[b] The mean number of *T. gondii* per infected cell and the mean number of *T. gondii* per vacuole 1 h after infection were not significantly different for control, treated peritoneal, or treated placental Mϕ (data not shown).

[c] Mean ± standard deviation.

[d] $P < 0.01$ compared to controls.

[e] $P < 0.05$ compared to controls.

TABLE 3. Effects of different IFN-γ preparations on survival and replication of *T. gondii* in Mϕ culture supplement[a]

IFN-γ prepn (U/ml)	Adult Mϕ		Placental Mϕ	
	% Mϕ infected (*n*)	$\bar{X}$ *T. gondii*/ vacuole	% Mϕ infected (*n*)	$\bar{X}$ *T. gondii*/ vacuole
Control	31.4 ± 19.1 (18)	5.8 ± 0.9	58.8 ± 25.6 (11)	6.2 ± 0.7
Standard rIFN-γ				
10	26.0 ± 15.8 (4)	5.0 ± 1.0	17.3 ± 3.2 (3)	3.0 ± 0.4
50	20.4 ± 10.0 (5)	3.8 ± 0.9	19.2 ± 9.7 (5)	2.8 ± 1.1
300	5.6 ± 3.7 (17)	2.7 ± 1.1	13.3 ± 8.7 (12)	2.2 ± 0.7
Modified rIFN-γ				
10	15.0 ± 0.0 (2)	5.1 ± 0.6	15.0 (1)	2.7
50	21.5 ± 10.6 (2)	5.1 ± 0.6	21.5 ± 16.0 (2)	3.3 ± 1.5
300	5.3 ± 4.5 (3)	1.8 ± 0.7	17.5 ± 19.1 (2)	2.2 ± 0.3
Purified rIFN-γ				
50	23.5 ± 12.7 (2)	4.4 ± 1.3	19.0 (1)	1.8
300	13.0 ± 13.9 (3)	2.6 ± 1.1	7.5 ± 0.7 (2)	1.9 ± 0.5
30% LK	10.2 ± 12.7 (10)	3.5 ± 1.2	25.0 ± 26.1 (4)	2.9 ± 1.2

[a] Mϕ were cultured in vitro for 4 to 5 days, and IFN-γ or LK was added for 72 h in the indicated concentration; then Mϕ were washed, infected with *T. gondii*, and evaluated as described in the text. The percentage of infected Mϕ and number of *T. gondii* per infected Mϕ at zero hour were similar to values in Table 1, footnote *a*. Results are expressed as mean ± standard deviation (*n*) at 20 h after infection.

5,401 ± 905 cpm for adult Mϕ ($n = 2$) and 41,767 and 8,051 cpm for placental Mϕ ($n = 1$), respectively; the protein contents of control and rIFN-γ-exposed Mϕ monolayers were similar.

Effects of different IFN-γ preparations and of rIFN-αA or rIFN-β on survival and replication of *T. gondii*. The different preparations of IFN-γ, both with adult monocyte-derived Mϕ and with placental Mϕ, were similar in their effectiveness when the amount of IFN-γ was expressed in antiviral units (Table 3).

rIFN-αA inhibited replication of *T. gondii* in adult monocyte-derived Mϕ and in placental Mϕ (Table 4). However, rIFN-αA significantly inhibited ($P < 0.05$) replication only at concentration of ≥300 U/ml, whereas rIFN-γ significantly ($P < 0.05$) inhibited replication at concentrations of ≥10 U/ml (Table 3); the optimum concentration of rIFN-γ (300 U/ml) was significantly ($P < 0.05$) more effective than was the optimal concentration of rIFN-αA (300 to 3,000 U/ml). Furthermore, rIFN-γ significantly inhibited survival of *T. gondii*, whereas rIFN-αA did not. rIFN-β was less active than was rIFN-αA or rIFN-γ and did not significantly increase the anti-*Toxoplasma* activity of Mϕ.

Effects of IFNs on survival and replication of *T. gondii* in WISH cells. Survival and replication of *T. gondii* in WISH cells was assessed by uptake of [^{3}H]uracil (Fig. 2). rIFN-γ inhibited [^{3}H]uracil uptake by *Toxoplasma*-infected WISH cells in a concentration-dependent manner. rIFN-αA and rIFN-β also inhibited [^{3}H]uracil uptake but were less effec-

TABLE 4. Effects of rIFN-αA and rIFN-β compared with rIFN-γ on survival and replication of *T. gondii* in Mϕ culture supplement

rIFN prepn (U/ml)	Adult Mϕ		Placental Mϕ	
	% Mϕ infected[a] (*n*)	$\bar{X}$ *T. gondii*/ vacuole[a]	% Mϕ infected (*n*)	$\bar{X}$ *T. gondii*/ vacuole
Control	33.3 ± 15.8[b] (6)	5.5 ± 0.7	53.2 ± 27.1 (5)	6.8 ± 1.0
rIFN-γ, 300	4.5 ± 3.4[c] (6)	2.1 ± 1.1[c]	10.2 ± 8.1[c] (5)	1.8 ± 0.3[c]
rIFN-αA				
10	27.7 ± 12.4 (3)	4.1 ± 0.5	ND[d]	ND
50	27.3 ± 19.8 (4)	4.0 ± 1.0	43.5 ± 9.2 (2)	4.7 ± 1.9
300	21.3 ± 9.0 (4)	3.0 ± 0.8[e,f]	43.3 ± 20.7 (3)	4.3 ± 1.4[e,g]
3,000	26.8 ± 7.9 (3)	2.8 ± 0.3[e,f]	42.0 ± 20.7 (3)	3.2 ± 1.4[e]
rIFN-β				
50	47.5 ± 2.1 (2)	4.5 ± 0.5	46.0 ± 27.5 (3)	5.1 ± 0.4[g]
300	62.0 ± 1.4 (2)	5.4 ± 0.4[e]	27.7 ± 29.8 (3)	4.3 ± 1.9
3,000	46.5 ± 10.6 (2)	4.2 ± 0.1	36.0 ± 36.8 (2)	3.8 ± 1.2

[a] Percentage of cells and mean number of *T. gondii* per cell were similar at zero hour in control and in treated Mϕ.
[b] Mean ± standard deviation at 20 h after infection. Results for controls and for rIFN-γ are pooled from experiments with rIFN-αA and rIFN-β. Statistical comparison was done by comparing results only from paired assays.
[c] $P < 0.01$ versus control.
[d] ND, Not done.
[e] $P < 0.05$ versus 300 U of rIFN-γ per ml.
[f] $P < 0.05$ versus control.
[g] $P < 0.01$ versus 300 U of rIFN-γ per ml.

tive than rIFN-γ. As previously reported (24, 27), uninfected cells did not incorporate the [^{3}H]uracil (<10 cpm/μg of protein).

DISCUSSION

These results indicate that IFN-γ effectively increases the ability of human Mϕ to kill *T. gondii* and to inhibit the replication of those parasites which survive. Purified and rIFN-γ were comparable in their effectiveness; crude LK, when diluted to a 30% final concentration, contained similar amounts of IFN-γ (~270 U/ml) and were similar in their effectiveness to the rIFN-γ or purified IFN-γ preparations. These results are similar to those reported by Nathan et al. (13, 14) with monocyte-derived Mϕ from adult humans and are consistent with their earlier studies (13, 14) and ours (24), which indicated that the ability of LK to activate Mϕ against *T. gondii* was completely eliminated by the addition of antibody to IFN-γ. The current studies extend these findings by showing for the first time that human tissue Mϕ, like monocyte-derived Mϕ, are activated by IFN-γ against *T. gondii* and that Mϕ from newborns, like Mϕ from adults, are activated by IFN-γ.

When infected with *T. gondii* immediately or within 18 h of isolation, both neonatal and adult blood monocytes restricted the intracellular replication of *T. gondii*, whereas peritoneal and placental Mϕ were less effective. Neonatal and adult monocytes and placental Mϕ became significantly more permissive for the survival and replication of *T. gondii* after they were cultured in vitro; peritoneal Mϕ became slightly but not significantly more permissive. Treatment with IFN-γ reversed this change so that the replication of *T. gondii* in IFN-γ-treated Mϕ was similar to that in freshly isolated monocytes and Mϕ. More strikingly, IFN-γ-treated Mϕ cleared *T. gondii* more effectively between 0 and 20 h of infection, as indicated by a greater decrease in the percentage of infected Mϕ, than did cultured or freshly isolated monocytes and Mϕ. Thus, IFN-γ-treated Mϕ had similar toxoplasmastatic activity and similar or greater toxoplasmacidal activity than do freshly isolated monocytes or Mϕ of the same type.

In contrast to the effects of IFN-γ, rIFN-αA only slightly increased the ability of adult or newborn Mϕ to inhibit *T. gondii* replication and did not increase the ability of these Mϕ to kill *T. gondii*. rIFN-β did not significantly inhibit survival and replication of *T. gondii* in Mϕ. These results are similar to those recently presented by Nathan et al. (14) with Mϕ derived from adult human monocytes. These authors also found that other cytokines present in crude LK, including interleukin 2, colony-stimulating factor, and Mϕ migration inhibition factor, did not activate Mϕ. Similarly, we have found that purified and recombinant interleukin 2 and purified interleukin 1 do not activate human Mϕ against *T. gondii* (unpublished data).

Since *T. gondii* infects constitutive cells as well as phagocytic cells, we examined the effects of IFNs on the survival and replication of *T. gondii* in WISH cells, the human cell line in which the antiviral activity of these IFN preparations was determined. [^{3}H]uracil uptake by *Toxoplasma*-infected WISH cells treated with rIFN-γ was inhibited in a concentration-dependent manner. These results parallel those of Pfefferkorn and Guyre (15). We found that although rIFN-αA and rIFN-β, were less effective than rIFN-γ, they weakly inhibited [^{3}H]uracil uptake by *Toxoplasma*-infected WISH cells. These results differ from those of Pfefferkorn and Guyre (15) and Ahronheim (1) in human fibroblasts, but are similar to those of Remington and Merigan (17) with mouse L cells and chicken embryo fibroblasts. The differences in results may reflect differences in IFN preparations, cell types used for the assays, or both. Nevertheless, it is clear that IFN-γ is the most potent factor described that enhances the anti-*Toxoplasma* activity of Mϕ and constitutive cells.

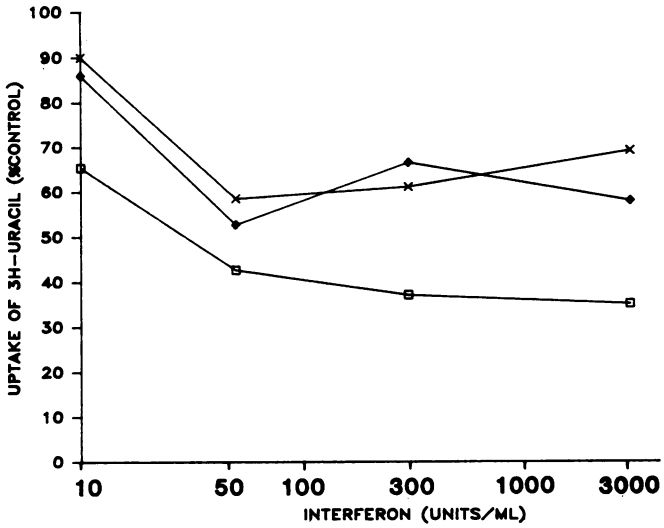

FIG. 2. Effects of rIFN-γ rIFN-αA, and rIFN-β on the survival and replication of *T. gondii* in WISH cells as determined by uptake of [^{3}H]uracil. WISH cells were exposed to the indicated concentration of rIFN-γ (□), rIFN-αA (◇), or rIFN-β (×) for 72 h and then infected with *T. gondii* for 1 h. [^{3}H]uracil uptake was determined 18 h later as described in the text. Results are the mean of five to seven experiments with rIFN-γ, three experiments with rIFN-αA, and two experiments with rIFN-β, except at concentrations of 3,000 U of rIFN-γ per ml and 10 U of rIFN-α and -β per ml (n = 1). Results for IFN-γ at 50 and 300 U/ml and rIFN-αA at 50 U/ml were significantly (P <0.05) different than controls.

These findings underscore the potential importance of IFN-γ production in the control of *Toxoplasma* infection. Stimulated lymphocytes from neonates produce less IFN-γ, with amounts varying from <10 to ~60% of that produced by cells of adults (2, 4, 23, 24; C. B. Wilson, unpublished data), whereas production of IFN-α (4, 16) by neonatal leukocytes and IFN-β (1) by neonatal fibroblasts is normal. IFN-γ effectively activates both newborn and adult Mϕ against *T. gondii* and inhibits the replication of *T. gondii* in constitutive cells derived from neonatal (foreskin fibroblasts) (15) and fetal (WISH cells) tissues. Thus, decreased production of IFN-γ, which would allow greater survival and replication of *T. gondii* in cells of neonates, may contribute to the susceptibility of the human fetus and neonate to severe *T. gondii* infection.

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