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Role of Beta Interferon in Resistance to *Toxoplasma gondii* Infection

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The role of recombinant murine beta interferon (rMuIFN-β) and recombinant human IFN-β (rHuIFN-β) in resistance to *Toxoplasma gondii* was examined. rMuIFN-β protected mice against a lethal infection with the parasite. The protective effect appeared to depend on the concomitant release of gamma interferon. rMuIFN-β did not activate murine peritoneal macrophages to inhibit or kill *T. gondii* whether used alone or in combination with lipopolysaccharide (LPS). rHuIFN-β did not activate human monocyte-derived macrophages to inhibit or kill *T. gondii* when 5-day-old monocyte-derived macrophages were used. In contrast, significant killing of *T. gondii* was noted when 10-day-old monocyte-derived macrophages were used. The addition of LPS enhanced this effect. These results revealed a role for IFN-β in the mechanisms of defense against *T. gondii* and suggest its potential use in the treatment of toxoplasmosis in humans.

In addition to its antiviral effects (7), the activity of beta interferon (IFN-β) has recently been extended to *Trypanosoma cruzi* (9) and *Listeria monocytogenes* (5). Experiments to demonstrate whether a similar effect can be demonstrated against *T. gondii* have revealed varied results (11, 17, 21). Because of the conflicting observations regarding the effect of IFN-β on *T. gondii* and the potential that IFN-β may have in the therapy of toxoplasmosis, particularly in immunocompromised patients, we considered it of interest to examine further the effect of this cytokine against this parasite both in vitro and in vivo.

Swiss Webster and BALB/c (Simonsen Laboratories Inc., Gilroy, Calif.) and CBA/Ca (The Jackson Laboratory, Bar Harbor, Maine) female mice were used to obtain tachyzoites (strain RH or C56) and cysts (strain ME49) of *T. gondii*, to obtain peritoneal macrophages, and for protection studies. For in vitro experiments, mouse peritoneal macrophages or human monocyte-derived macrophages (MDM) were used. Murine macrophages were incubated overnight at 37°C with various concentrations of recombinant murine IFN-β (rMuIFN-β; endotoxin, 0.02 ng/10^6 U; Toray Industries, Inc., Kanagawa, Japan) in triplicate wells. Thereafter, the cells were infected with RH tachyzoites (10). One hour later, extracellular parasites were removed, and the monolayers were reincubated with medium for various periods of time, washed, fixed, and stained with Giemsa stain. Activity was evaluated by determining percent cells infected and the number of tachyzoites per 100 infected cells. In some experiments cells were exposed to various concentrations of rMuIFN-β combined with lipopolysaccharide (LPS; *Escherichia coli* O26:B6 [Difco Laboratories, Detroit, Mich.]) at a concentration of 0.1 ng/ml. MDM were obtained by culturing peripheral blood mononuclear cells (1) obtained from healthy adult donors. Greater than 95% of the cells stained for nonspecific esterase (21) and were used after either 5 or 10 days of culture. The MDM were incubated with various concentrations of recombinant human IFN-β (rHuIFN-β; endotoxin, <0.01 ng/ml; Triton Biosciences, Alameda, Calif.) alone or in combination with LPS (0.1 ng/ml). RH tachyzoites were used for infection (20).

To determine the in vivo protective effect of rMuIFN-β, various concentrations of this cytokine were prepared in preservative-free sodium chloride solution (Elkins-Sinn, Inc., Cherry Hill, N.J.) and injected intravenously 3 h prior to infection with *T. gondii* tachyzoites of strain C56. Control mice were injected with preservative-free saline alone. In addition, an optimal concentration of a monoclonal antibody to murine gamma interferon (anti-MuIFN-γ) was used to abrogate the protective effect of endogenous IFN-γ against *T. gondii* (10). The anti-IFN-γ monoclonal antibody was injected intraperitoneally at a concentration of 0.1 mg. Mortality data were analyzed by using the chi-square test, and differences in rates of intracellular infection and replication of *T. gondii* in macrophages were analyzed with Student's *t* test.

The in vitro results with murine macrophages revealed that neither rate of infection nor replication of *T. gondii* within macrophages was altered by rMuIFN-β (Table 1). Similarly, when MDM obtained by culturing human peripheral blood mononuclear cells for 5 days were used, differ-

TABLE 1. Activity of rMuIFN-β on replication of *T. gondii* within mouse peritoneal macrophages[a]

Group	% Cells infected		Parasites per 100 infected cells	
	1 h	18 h	1 h	18 h
Untreated	47 ± 3.1	46 ± 2.9	135 ± 4.5	440 ± 4.3
rMuIFN-β treated (U/ml)				
10	47 ± 2.7	43 ± 3.2	174 ± 4.1	491 ± 6.3
10^2	43 ± 3.6	45 ± 4.7	166 ± 5.3	501 ± 4.8
10^3	43 ± 2.8	38 ± 3.1	190 ± 3.8	390 ± 3.7
10^4	46 ± 3.9	42 ± 3.3	170 ± 4.3	462 ± 5.1
10^5	ND	40 ± 4.3	ND	392 ± 3.9

[a] Values are means ± standard errors of triplicate monolayers in a representative experiment. ND, not determined.

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TABLE 2. Effect of rHuIFN-β on intracellular replication of *T. gondii* in human MDM cultured for 10 days[a]

Treatment	Cells infected[b]		Parasites[b]	
	%	*P*	No. per 100 infected cells	*P*
None	44 ± 3.7		670 ± 13.3	
rMuIFN-β (U/ml)				
10	39 ± 5.8 (11)[c]	>0.05	490 ± 5.5 (27)	>0.05
10^2	30 ± 2.6 (32)	<0.025	446 ± 2.1 (33)	>0.05
10^3	22 ± 2.6 (50)	<0.01	370 ± 4.7 (45)	>0.05

[a] Values are means ± standard errors of triplicate monolayers of a representative experiment of three performed.
[b] Determined 18 h after infection of cells.
[c] Values in parentheses are percent reduction compared with untreated monolayers.

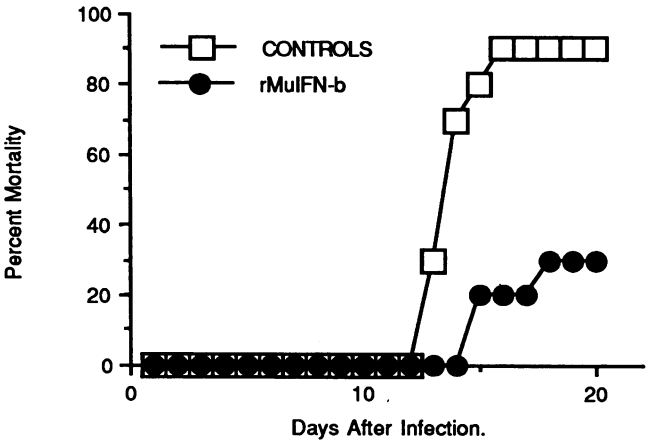

FIG. 1. Protective activity of rMuIFN-β in mice injected intravenously with 10^4 U of the cytokine and infected intraperitoneally with 10^2 tachyzoites of *T. gondii* C56.

ences in the rates of infection or replication of *T. gondii* in treated and control monolayers were not noted (data not shown). In contrast, when MDM were cultured for 10 days prior to use, the numbers of intracellular tachyzoites in rHuIFN-β-treated cells decreased significantly (Table 2). Addition of LPS to the MDM cultures significantly enhanced the ability of IFN-β to activate the antitoxoplasma activity of MDM (Table 3). LPS alone did not have any effect.

The experiments performed in vivo revealed that injection with 10^4 U of rMuIFN-β protected mice challenged with 10^2 tachyzoites of *T. gondii* C56 (Fig. 1) but not mice challenged with 10^3 or 10^4 tachyzoites (data not shown). At 21 days postinfection, mortality in the rMuIFN-β-treated group was 30% compared with 90% in the control group ($P \leq 0.01$) (Fig. 1). Because previous work identified IFN-γ as a major mediator of resistance against *T. gondii* in the mouse (19), we considered it of interest to determine whether administration of rIFN-β affected endogenous production of IFN-γ in the infected and control mice. A monoclonal antibody to IFN-γ was used to ablate endogenous production of IFN-γ. Mice were injected with this monoclonal antibody alone, rMuIFN-β alone, or monoclonal anti-IFN-γ in combination with 10^4 U of rMuIFN-β on days −1 and +6 of infection with 40 cysts of *T. gondii* ME49. rMuIFN-β was administered 3 h before infection. At the end of the experiment (21 days of infection), 80% of the control and only 20% of the rMuIFN-β-treated mice had died ($P \leq 0.01$) (Fig. 2). In contrast, all mice treated with monoclonal antibody to IFN-γ alone or in combination with rMuIFN-β were dead by day 9 of infection ($P \leq 0.001$) (Fig. 2).

The results described above revealed that administration of IFN-β induced significant resistance against two different strains of *T. gondii*. The resistance appeared to be associated with the production of IFN-γ since treatment of mice with a monoclonal antibody against IFN-γ abrogated the protective effect of IFN-β, suggesting that IFN-β alone may not be sufficient to protect against acute *T. gondii* infection in the murine model. It is likely that one of the in vivo actions of IFN-β may be related to its ability to stimulate production of IFN-γ (13) and consequent activation of macrophages to inhibit or kill *T. gondii*. rMuIFN-β has been reported to enhance peroxide release from mouse peritoneal macrophages in vivo but not in vitro, and some of its protective effects against *L. monocytogenes* infection in mice have been explained in this context (5). Effects of IFN-β in vivo other than stimulation of IFN-γ production may also have contributed to the enhanced resistance of the recipient animals. IFN-β activates NK cells (14), which in one report (6) were shown to kill *T. gondii* tachyzoites. In addition, murine IFN-β has been reported to induce NK activity in cloned mouse cytotoxic T lymphocytes (2). Whether IFN-β can enhance cytotoxic T-lymphocyte activity against *T. gondii* is not known, but killing activity against *T. gondii* has been demonstrated with cytotoxic T lymphocytes (8, 22). Also, IFN-β has been documented to induce the synthesis or release of tumor necrosis factor from adherent human bone marrow accessory cells (15). Of interest was the demonstration of in vivo inhibition of murine suppressor T lymphocytes by IFN-β (16). Whether such inhibition may have contributed to resistance against *T. gondii* during the acute infection is unclear. The results of the present study must be interpreted in light of the fact that IFN-γ and/or IFN-α/β production is induced in mice acutely infected with *T. gondii* (3, 4, 18). Thus, it is possible that these cytokines may be produced sufficiently early in the infection to enhance the effect of exogenously administered IFN-β.

TABLE 3. Effect of rHuIFN-β alone or in combination with LPS on anti-*T. gondii* activity of human MDM cultured for 10 days[a]

Treatment	Cells infected at given rHuIFN-β concn[b]					Parasites at given rHuIFN-β concn[b]				
	%		*P*	% at 5×10^3 U	*P*	No. per 100 infected cells		*P*	No. per 100 infected cells at 5×10^3 U	*P*
	0	2×10^3 U				0	2×10^3 U			
None	38 ± 2	26 ± 2.5 (31)[c]	<0.05	30 ± 2 (22)	<0.02	564 ± 27	311 ± 9 (38)	<0.05	275 ± 28 (47)	<0.002
LPS (1 ng/ml)	41 ± 1.5	15 ± 1.5 (42)	<0.025	17 ± 0.9 (43)	<0.05	448 ± 6	168 ± 14 (46)	<0.01	158 ± 1 (43)	<0.01

[a] Values are means ± standard errors of triplicate monolayers of a representative experiment of three performed.
[b] Determined 24 h after infection.
[c] Values in parentheses are percent decrease compared with the respective control.

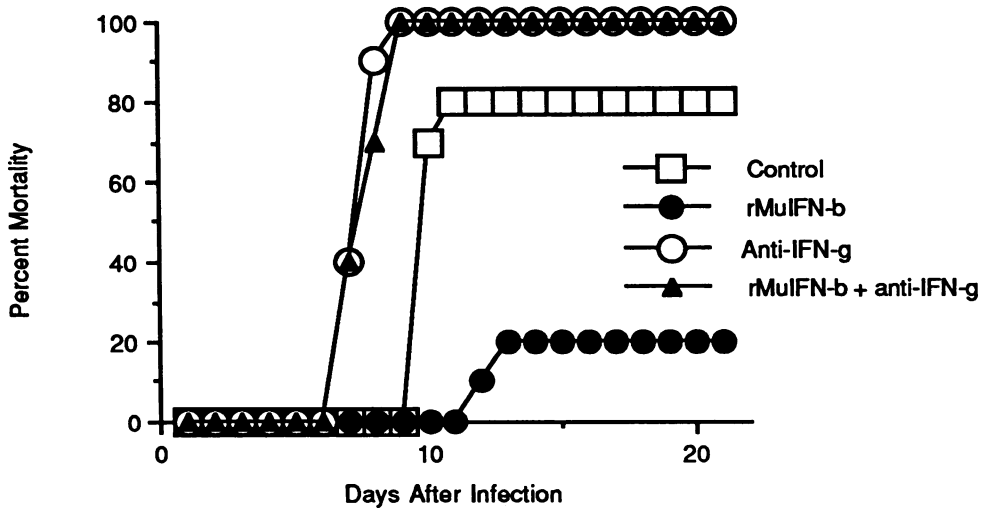

FIG. 2. Monoclonal antibody to IFN-γ abrogates the protective activity of rMuIFN-β against infection with tissue cysts of *T. gondii* in mice.

Previous studies to define whether rIFN-β can confer upon human macrophages the capacity to inhibit or kill *T. gondii* tachyzoites have given conflicting results (11, 12, 17, 21). In some studies minimal or no effect was noted (11, 12, 21), whereas in others a definite effect was reported (17) which appeared to depend upon the anatomical site from which the macrophages were obtained and the duration of MDM in culture. Our results revealed that treatment with IFN-β alone did induce significant activation of MDM against *T. gondii*. We found that multiplication of *T. gondii* in MDM cultured for 5 days and then treated with IFN-β did not differ from controls, whereas there was significant killing of tachyzoites in those cells cultured for 10 days prior to treatment with IFN-β. Moreover, treatment with LPS further enhanced this effect. Our results suggest that the duration of MDM in culture is a critical factor for induction of antitoxoplasma activity by rHuIFN-β. This may explain the apparently contradicting results reported previously by other investigators (11, 12, 17, 21).

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