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Interferon: Protection of Cells Infected with an Intracellular Protozoan (Toxoplasma gondii)

Abstract. *Chick and mouse cell monolayers treated with interferon were significantly protected from destruction by toxoplasma. The results suggest that fewer numbers of organisms were released from interferon-treated cells. Interferons were uniformly more active in a viral assay than in a toxoplasma assay, and they had the same properties in both assays. The data suggest that interferon activity may be directed against organisms phylogenetically higher than viruses.*

A significant role for interferon in protection of cells from destruction by obligate intracellular parasites other than viruses is suggested by the observations on its effect in infections with organisms such as psittacosis (*1*) and trachoma-inclusion conjunctivitis (TRIC) agents (*2*, *3*). In that the obligate intracellular protozoan, *Toxoplasma gondii,* induces interferon production (*4*), we set out to determine if treatment of cells in vitro with interferon will alter their resistance to destruction by toxoplasma.

Chick interferon was prepared in 9- to 11-day-old embryonated eggs with the WS strain of influenza (*5*), and mouse interferon was prepared in L cells with Newcastle disease virus (*2*). Antiviral activity of mouse and chick interferon was measured in L cell and chick embryo fibroblast monolayers, respectively, with the use of bovine vesicular stomatitis virus (VSV) (*5*). For evaluation of mouse interferon activity against toxoplasma, 6×10^5 L cells that had been grown in monolayers were suspended in 2.4 ml of various dilutions of interferon in Eagle's minimal essential medium (MEM) supplemented with inactivated fetal calf serum (10 percent), streptomycin (100 μg/ml), and penicillin (50 unit/ml). The fetal calf serum used contained no toxoplasma antibodies, as judged by the Sabin-Feldman dye test. The cell suspensions (0.4 ml) were plated on cover slips (22 mm) attached to supporting glass rings with silicone grease and incubated at 37°C for 16 hours in an atmosphere containing 5 percent of CO_2. After the monolayers were washed twice with Earle's balanced salt solution, 0.5 ml of supplemented MEM containing 5×10^5 toxoplasma was placed on each monolayer (approximately 2.5 organisms per cell). The RH strain of toxoplasma was used in all experiments and was obtained from the peritoneal fluid of mice that had been infected 3 days earlier. The monolayers were then incubated at 37°C for 1 hour, washed with Earle's solution and incubated again in supplemented MEM at 37°C for varying periods of time. Monolayers were fixed in Bouin's solution and were stained with hematoxylin and eosin. Destruction of an interferon-treated monolayer was determined quantitatively by comparison to both uninfected monolayers (value of 0) and untreated infected monolayers (value of 4+). The intermediate degrees of destruction were scored with a value of 1+ for 10 to 30 percent destruction, 2+ for >40 to 70 percent destruction, and 3+ for 75 to 90 percent destruction.

Activity of chick interferon against toxoplasma was tested by a plaque assay in secondary cultures of chick embryo fibroblasts. Cells (5×10^5) were seeded in petri dishes (60 by 15 mm) in a modification (fetal calf serum was used) of the supplemented medium 199 described by Rubin (*6*). When the monolayers were confluent (usually 48 hours), the medium was removed and replaced with 4 ml of the supplemented medium or with dilutions of interferon in this medium. The monolayers were incubated for 8 hours, and washed with Earle's solution, and approximately 300 toxoplasma in 2 ml of suspending medium (*7*) were added to each dish. After incubation for another hour, the medium was removed and replaced with overlay medium (*8*). After 5 days at 37°C in an atmosphere containing 5 percent of CO_2 the cells on the plates were stained with a solution (0.05 percent) of neutral red, and plaques were counted. Microscopic examination revealed the plaque areas to consist of cell debris and free toxoplasma.

Table 1. Assay of interferon activity in L cells with vesicular stomatitis virus and toxoplasma.

Interferon preparations	Activity	
	VSV*	Toxoplasma †
A. Pressure dialyzed crude	1/20,000	1/400
B. Crude (experiment 1)	1/900	1/50
Crude (experiment 2)	1/900	1/50
C. CM-Sephadex purified	1/2200	1/300
D. Lyophilized CM-Sephadex purified ‡	<1/30	<1/10
E. XE-64 purified (with 0.05 percent bovine serum albumin added)	1/560	1/20
F. XE-64 purified	1/275	1/30

* Dilution of interferon preparation which inhibits VSV plaque formation in L cells by 50 percent. † Dilution of interferon preparation which reduces L cell destruction by toxoplasma from 4+ in control-infected monolayer to 2+ destruction. ‡ The original titer of this preparation was 1:100 against VSV and, in contrast to the usually observed stability of such lyophilized preparations, had markedly decreased on storage—this loss in activity was reflected by results obtained in both assays.

Monolayers of L cells treated with mouse interferon before infection were significantly protected from destruction by toxoplasma as compared with untreated infected monolayers (Fig. 1). However, in all interferon-protected monolayers there were intracellular replicative forms of toxoplasma. A preparation of chick interferon which was active in the chick cell plaque-reduction assay with toxoplasma showed no activity in the assay consisting of toxoplasma and L cells. Mouse interferon was not active against toxoplasma infection as judged in a cover slip assay with chick embryo fibroblasts rather than L cells. Samples treated with mouse interferon at various temperatures for 1 hour were stable at 4° and 37°C, partially lost their activity at 56°C, and completely lost activity at 80°C in L cell assay both with virus and with toxoplasma. In both assays lowering of the *p*H to 2 did not destroy interferon activity; the interferon activity did not sediment after cen-

trifugation for 2 hours at 90,000*g*. Dialysis for 18 hours against the maintenance media did not result in any measurable loss of activity; treatment with trypsin destroyed all measurable activity. Several preparations which had been purified by gradient ion-exchange column chromatography on either the Amberlite XE-64 resin or carboxymethyl- (CM) Sephadex were tested for their activity against both VSV and toxoplasma (Table 1). The titers in the toxoplasma assay are approximations since the method for scoring survival of toxoplasma-infected monolayers is less quantitative than the VSV plaque-reduction assay. The interferon preparations were uniformly more active in the virus assay than against toxoplasma. There is apparently a direct relation between the protective effect of the various interferon preparations in the VSV assay and their protective effect in the toxoplasma assay, and all characteristics of the inhibitor fit those described for interferon (*9*).

Three experiments in vivo revealed that greater numbers of organisms are released from untreated monolayers than from monolayers treated with interferon (Table 2), as might be expected from the protective effect of interferon on these same monolayers. These experiments were chosen to measure overall interferon effect rather than its effect on all the morphologically identifiable intracellular stages of development. Mice were inoculated with varying log dilutions (10^{-3} to 10^{-6}) of a pool of equal volumes of media obtained from two monolayers which had been incubated for 12 or 24 hours after toxoplasma infection. Those inoculated with media from monolayers that had been treated with interferon showed a significantly lower mortality and the time to death was longer when compared with mice inoculated with the same dilutions of media from untreated monolayers. Studies of the monolayers by light and electron microscopy might be helpful in determining whether an alteration in multiplication and/or morphology of toxoplasma can be observed in cells treated with interferon.

Table 2. Assay in vivo for release of toxoplasma from interferon-treated and untreated monolayers. Mice were inoculated with dilutions of medium obtained from monolayers which had been incubated for 12 or 24 hours after infection.

Dilutions of medium	Time after infection			
	12 hours		24 hours	
	Day of death (mean)	Dead (%)	Day of death (mean)	Dead (%)
	Control			
10^{-3}*	8.8	80	9.0	100
10^{-4}	9.3	60	10.0	80
10^{-5}	10.0	20	9.0	20
10^{-6}			9.0	20
	Interferon treated			
10^{-3}	10.0	40	9.3	60
10^{-4}	9.0	20	19.0	20
10^{-5}		0		0
10^{-6}				0

* Five mice inoculated with each dilution.

Plaque reduction (50 percent or greater) occurred in plates of chick embryo fibroblasts treated with chick interferon. A representative example of the plaque count with dilutions of chick interferon ranging from 1:2 to 1:16 were: controls 17, 18, 25, 35; 1:2, 4, 5, 7; 1:4, 10, 11, 11; 1:8, 13, 14, 16; and 1:16, 14, 18. Experiments with serial dilutions of some of the preparations of chick interferon revealed that there was a direct relation between interferon concentration and plaque-reducing activity. In three experiments with two different preparations of interferon the ratio of viral plaque-reduction activity to toxoplasma plaque-reduction activity ranged from 10:1 to 20:1. Activity of the preparations as measured in both viral and toxoplasma assay systems was destroyed or significantly reduced by treatment with trypsin, was partially destroyed after incubation at 56°C for 1 hour, and was not reduced by dialysis overnight against maintenance media. Treatment of chick-cell monolayers with mouse interferon which was active in the L cell system did not result in plaque reduction. Shortening the period of time of incubation of interferon with the monolayers to 1 hour significantly decreased its plaque-reducing activity.

The mechanism of interferon action appears to be mediated by failure of the host cell ribosomes to translate viral messenger RNA (*10*). It may be that one can extrapolate directly from the action of interferon on cells infected with virus to its mechanism of action on cells infected with phylogenetically higher organisms. Whereas viruses lack many of the components necessary for protein synthesis and thus must depend on host cell components for replication, the reason for the necessity of an intracellular habitat for toxoplasma is unknown. Electron microscopy indicate that toxoplasma have a well-defined nucleus, Golgi apparatus, mitochondria, and endoplasmic reticulum with what appears to be ribosomes (*11*). Studies on RNA isolated from toxoplasma indicated a unique ribosomal RNA which could be differentiated from ribosomal RNA of the host cell (*12*). If toxoplasma depend on their own ribosomes for protein formation, the interferon effect we have observed might result

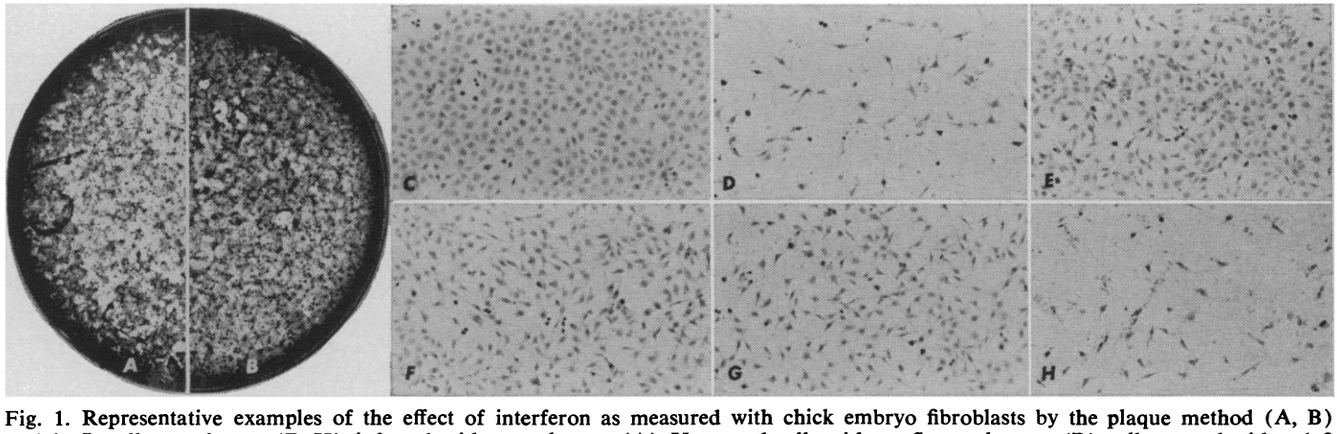

Fig. 1. Representative examples of the effect of interferon as measured with chick embryo fibroblasts by the plaque method (A, B) and in L cell monolayers (D–H) infected with toxoplasma. (A) Untreated cells with confluent plaques; (B) cells treated with a 1:2 dilution of chick interferon; (C) L cell control monolayer; (D) untreated monolayers. Monolayers E through H received the following dilutions of mouse interferon (preparation C in Table 1), respectively: E, 1:20; F, 1:40; G, 1:300; H, 1:1000. The L cells had been incubated for 24 hours after infection with toxoplasma.

from an action of the "antiviral" protein (*13*) on the ribosomes of toxoplasma which interferes with translation of toxoplasma messenger RNA by toxoplasma ribosomes. Among alternative explanations is the possibility that host cell ribosomes are used in toxoplasma replication, and in the interferon-treated cells they fail to translate toxoplasma messenger RNA. Either of these mechanisms may account for the observed action of interferon on cells infected with psittacosis (*1*) and TRIC agents (*2, 3*). Certainly the current definition of interferon in terms of its antiviral properties alone must be broadened to include its action against phylogenetically higher organisms.

Variations in virulence of certain viruses appears to be correlated with the ability to induce interferon production (*14*). The wide variation in virulence among different strains of toxoplasma (*15*) may, in a similar manner, be related to their ability to induce interferon production. As toxoplasma have been shown to stimulate interferon production, the role of interferon as a determinant in recovery from protozoal intracellular infection (such as toxoplasma, malaria, leishmania) in vivo must be considered. The general nature of interferon action against intracellular protozoa and its possible role in vivo are suggested by the independent findings of Jahiel *et al.* (*16*) on the protective effect of interferon-inducing agents in the mouse against *Plasmodium berghei* infection.

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8. Each 100 ml contained 55 ml of medium 199, 20 ml of medium 199 (double strength), 2 ml of tryptose phosphate broth, 1 ml of fetal calf serum, 1 ml of chick serum, 1 ml of a 2.8-percent solution of sodium bicarbonate and 20 ml of a 4.75-percent solution of Noble special agar (Difco).
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Deoxyribonucleic Acid Antibody: A Method to Detect Its Primary Interaction with Deoxyribonucleic Acid

Abstract. *Antibody to DNA in human serums can be detected by the ammonium sulfate method. This sensitive and specific technique, which measures the primary interaction between DNA and antibody to DNA, is based on the observation that free DNA is soluble in 50-percent saturated ammonium sulfate whereas antibody-bound DNA is insoluble.*

Antibodies specific for DNA in the serums of patients with systemic lupus erythematosus have been demonstrated by several immunologic techniques including quantitative precipitation, immunodiffusion in agar, complement fixation, and hemagglutination (*1*). These techniques measure a secondary manifestation which may or may not occur after a primary reaction between antigen and antibody has taken place. Since some serums, especially in the human, have low precipitating, complement-fixing, or hemagglutinating efficiencies (*2*), a technique which measures the primary interaction between DNA and antibody should provide information indicative of total antibody. We now report on a primary binding technique to measure DNA antibody by the ammonium sulfate method which was originally described for bovine serum albumin (*3*). Antibody-bound DNA can be separated from free DNA by this technique because DNA is soluble in 50-percent saturated ammonium sulfate solutions, whereas DNA-antibody complexes are insoluble.

Human serums were obtained from patients with systemic lupus erythematosus and from normal subjects. All patients' serums had antibodies to nuclear material as demonstrated by the indirect immunofluorescent technique; some serums had precipitins to DNA as detected by immunodiffusion in 0.4 percent agarose against calf thymus DNA (*4*). None of the normal serums had demonstrable DNA precipitins or antibodies to nuclear material.

Tritiated DNA (H^3-DNA) was prepared by growing *Bacillus subtilis* in medium (*5*) containing tritiated adenine or thymidine (*6*) and was isolated and purified as reported (*7*). The ammonium sulfate test has been described (*3*). DNA from calf thymus (20 μg in 1 ml of borate buffer at *p*H 8.4; 0.1 ionic strength) or H^3-DNA from *B. subtilis* (2 μg in 0.5 ml borate buffer) were added to equal volumes of serum diluted 1 :10 in borate buffer. Subsequent dilutions of serums were made

Table 1. DNA binding by normal human serums and systemic lupus erythematosus serums. Results are expressed as the percentage of calf thymus DNA or *Bacillus subtilis* tritiated DNA (H^3-DNA) detected by diphenylamine or isotopic assay in the 50-percent saturated ammonium sulfate precipitates (ppt) of the serums diluted 1:10. DNA precipitins were detected in patients' serums Nos. 1 to 4 but not in the remaining patients' or normal human serums.

Serums (No.)	20 μg DNA (% in ppt)	2 μg H^3-DNA (% in ppt)
Normal human		
1	7.5	3.7
2	2.5	2.3
3	7.5	2.0
4	5.0	1.4
5	2.5	2.1
6	7.5	4.5
7	5.0	8.8
8	5.0	1.7
9	7.5	2.8
Pool	2.5	2.6
Systemic lupus erythematosus		
1	97.5	71.7
2	77.5	57.2
3	72.5	51.6
4	15.0	25.3
5	10.0	10.7
6	10.0	14.1
7	7.5	5.0
8	5.0	5.0