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Protection against *Toxoplasma gondii* in Mice Immunized with *Toxoplasma* Cell Fractions, RNA and Synthetic Polyribonucleotides

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Summary. Mice immunized with fractions obtained by centrifugation of disrupted *Toxoplasma gondii* trophozoites as well as with 200 μg of *Toxoplasma* ribonucleic acid (RNA) were resistant (as measured by time to death and total mortality) to challenge with *Toxoplasma* 30 days later. When mice were challenged at 15 days no protection was noted. A dose of 50 μg of *Toxoplasma* RNA was effective in protecting mice against lethal challenge only when incorporated into Freund's incomplete adjuvant. In studies performed to determine the specificity of the resistance observed, resistance was also noted in mice immunized with 200 μg of RNA extracted from normal mouse peritoneal macrophages, as well as in mice immunized with 100 μg of the synthetic polyribonucleotide polycytidylic acid. Polyadenylic–uridylic acid conferred protection only when incorporated into Freund's incomplete adjuvant and polyinosinic–cytidylic acid had no effect. The protection induced by *Toxoplasma* RNA was eliminated by prior treatment of the preparation with ribonuclease but not by treatment with pronase, suggesting that the moiety responsible for the protective effect was RNA. In experiments designed to explore the mechanism of resistance in the vaccinated mice, macrophages harvested from mice which had been injected with *Toxoplasma* RNA 15 days earlier were found to be activated in that they resisted challenge with *Listeria monocytogenes*.

INTRODUCTION

The report by Youmans and Youmans (1965) that ribosomes and ribonucleic acid (RNA) extracted from *Mycobacterium tuberculosis* confer resistance in mice to the tubercle bacillus has led to the investigation of the potential protective effect of these preparations against several species of bacteria. Within the past few years a number of reports dealing with the immunizing activity of ribosomes, whole cell RNA, ribosome-associated RNA, and ribosome-isolated protein have appeared in the literature (Johnson, 1972; Thomas and Weiss, 1972; Venneman, Bigley and Berry, 1970; Winston and Berry, 1970). It is generally agreed that ribosome and whole cell RNA are effective in inducing resistance in mice against a challenge with virulent strains of the bacterial species from which they were

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prepared. There is, however, controversy as to the immunogen involved in this effect, whether RNA itself (Venneman and Bigley, 1969; Venneman *et al.*, 1970; Youmans and Youmans, 1966), RNA associated to a protein moiety (Winston and Berry, 1970b), or protein alone (Johnson, 1972). Differences in the methods employed to obtain and purify RNA and the fact that different species of bacteria have been involved in the studies may account for the discrepancies noted in some reports (Johnson, 1972; Venneman *et al.*, 1970). Venneman *et al.* (1970) and Smith and Bigley (1972) have suggested that the mechanisms by which mice immunized with RNA preparations resist ordinarily lethal challenge is related to cellular immunity. However, we were unable to find data on the specificity of the protective effect induced by the various preparations.

The purpose of the present study was to determine whether ribosomes and RNA of an obligate intracellular protozoan, *Toxoplasma gondii*, can confer resistance against challenge with a virulent strain of this same organism. When this was demonstrated, further studies were performed to evaluate the specificity of the protective effect afforded by the RNA preparations and to determine if the RNA extract might confer resistance through activation of macrophages.

MATERIALS AND METHODS

Preparation of Toxoplasma *cell fractions*

Purified preparations of *Toxoplasma gondii* of the RH strain were obtained by filtration of peritoneal fluid of infected mice as previously described (Remington, Bloomfield, Russel and Robinson, 1970). Complete disruption of the organism was accomplished by sonicating the purified organisms in distilled water for 4 minutes with the power source set at 60 cycles using the intermediate probe BP 111 12T (Biosonik sonicator, Bronwill Scientific Incorporated, Rochester, New York). The sonicated material was centrifuged for 10 minutes at 300 ***g*** to sediment coarse cellular debris. The supernatant was then centrifuged for 15 minutes at 20,000 ***g*** and the sediment separated as fraction 1. The supernatant was again centrifuged for 20 minutes at 41,535 ***g*** andthe sediment separated as fraction 2. The remaining supernatant was centrifuged for 4 hours at 106,000 ***g*** and the sediment separated as fraction 3.

Extraction of Toxoplasma *RNA*

Filtered *Toxoplasma* of the RH strain (Remington *et al.*, 1970) were resuspended in Tris buffer (Tris–HCl, pH 7·5, 0·01 M; NaCl 0·1 M; EDTA 0·001 M) and a volume of a 20 per cent solution of sodium dodecyl sulphate (SDS) was added to effect a final SDS concentration of 1 per cent. The suspension in a glass-stoppered centrifuge tube was agitated and allowed to stand for 30 minutes at room temperature. RNA was extracted with aqueous phenol and ethyl alcohol as previously described (Remington *et al.*, 1970). The same method was used to extract RNA from macrophages harvested from normal mice.

The concentration of RNA in the preparations was determined by measuring the absorbancy at 260 nm and by the orcinol reaction (Williams and Chase, 1968). Characterization of RNA was performed by sucrose density gradient ultracentrifugation in a gradient of 5–20 per cent sucrose as previously described (Remington *et al.*, 1970). Protein determinations were performed by the biuret reaction using bovine serum albumin as the standard. The ribosomal preparation had approximately 30 per cent protein and the

phenol-extracted RNA has 1 per cent. The presence of ribosomes in the sediment obtained after centrifugation at 106,000 rev/min was determined by electron microscopy.

Enzymatic treatment of ribosomes and Toxoplasma *RNA*

Ribosomes and RNA were treated with deoxyribonuclease (Worthington Biochemical Corporation, New Jersey) to eliminate deoxyribonucleic acid (DNA) which might be present in the preparations (Venneman *et al.*, 1970). Pronase and ribonuclease (Calbiochem) were employed in a concentration of 100 μg of enzyme per 1000 μg of RNA and the mixture was incubated at 37° for 60 minutes (Johnson, 1972). The digest was dissolved in Tris buffer such that 0·2 ml contained 200 μg of RNA.

Mice

Six-week-old female mice of the Swiss–Webster strain were used (Horton Laboratories Incorporated, Oakland, California). The percentage survival was calculated to the nearest whole number and significance of differences determined by the chi-square test.

Immunization of mice with Toxoplasma *cell fractions*

Groups of mice were injected intraperitoneally (i.p.) with 200 μg wet weight/mouse of either fractions 1, 2 or 3. The material inoculated was in 0·2 ml containing, by volume, 50 per cent fraction in phosphate-buffered saline (PBS) and 50 per cent Freund's incomplete adjuvant (FIA), or 50 per cent PBS when FIA was not used. The supernatant of fraction 3 was injected in the same manner. Two groups of mice were used as controls; one injected with FIA alone and the other with 0·2 ml of PBS.

Immunization with Toxoplasma *RNA*

Two doses of RNA were used, 50 μg and 200 μg. When FIA was used, the RNA was mixed with an equal volume of the adjuvant. The inocula were administered i.p. in a volume of 0·2 ml.

Immunization with synthetic polyribonucleotides

Three synthetic polyribonucleotides were employed: polyinosinic–cytidylic acid (poly I:C); polyadenylic–uridylic acid (poly A:U); and polycytidylic acid (poly C) (Miles Laboratories, Elkhart, Indiana). They were administered mixed with FIA or PBS. The i.p. dose was 5 mg of polyribonucleotide per kilogram of body weight.

Toxoplasma *challenge*

Fifteen or 30 days after immunization, mice were challenged i.p. with 5×10^4 *Toxoplasma* trophozoites of the C_{56} strain and followed for time to death. This challenge dose gave an approximately LD_{70} at 20 days. Three days prior to challenge three mice from each group were bled and their sera tested for the presence of *Toxoplasma* antibody using the Sabin–Feldman dye test as described by Frenkel and Jacobs (1958).

Evaluation of peritoneal macrophages

Macrophage monolayers were prepared, challenged with *Listeria monocytogenes*, and evaluated as previously described (Ruskin and Remington, 1971).

RESULTS

EFFECT OF FRACTIONS OF DISRUPTED TOXOPLASMAS

As can be seen in Table 1 and Fig. 1, all fractions administered with or without FIA were effective in protecting mice against *Toxoplasma* challenge. Whereas 93 per cent of

TABLE 1
IMMUNIZING EFFECT OF *Toxoplasma* CELL FRACTIONS

Immunization	Amount (μg)	Freund's incomplete adjuvant	Percentage surviving for 20 days (number surviving/total)	*P* value
Fraction 1	200	No	93 (14/15)	<0·001
Fraction 2	200	No	93 (14/15)	<0·001
Fraction 3	200	No	67 (8/12)	0·02
Controls	—	No	24 (5/21)	—
Fraction 1	200	Yes	94 (17/18)	<0·001
Fraction 2	200	Yes	88 (15/17)	0·008
Fraction 3	200	Yes	94 (15/16)	0·003
Controls	—	Yes	35 (7/20)	—

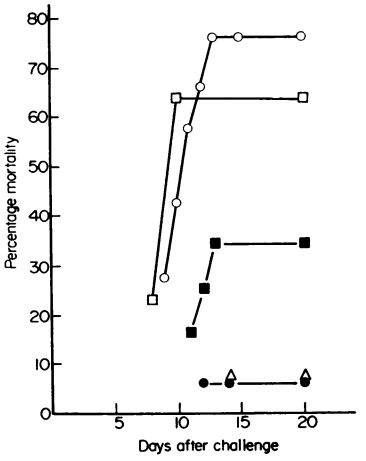

FIG. 1. Mortality in mice immunized with cell fraction and supernatant of disrupted *Toxoplasma* trophozoites without adjuvant. (○) Controls. (□) Supernatant. (△) Fraction 1. (●) Fraction 2. (■) Fraction 3.

mice injected with fraction 1 or fraction 2 without FIA survived, only 24 per cent of controls survived ($P<0·001$). Sixty-seven per cent of mice injected with fraction 3 survived ($P = 0·02$). FIA increased markedly the immunizing effect of fraction 3 ($P = 0·02$ without FIA to $P = 0·003$ with FIA). The supernatant of fraction 3, when administered with or without FIA, did not confer a significant protective effect.

Three mice from each group were tested for antibodies to *Toxoplasma* and all but the ones injected with the supernatant of fraction 3 were positive. The titres ranged from

1:64 to 1:128 for those injected with fractions 1 and 2 and from 1:16 to 1:64 for those injected with fraction 3.

EFFECT OF *Toxoplasma* RNA

Four experiments were performed and the results were similar in each. Representative results using RNA in Tris buffer are shown in Table 2. A significant protective effect

TABLE 2

IMMUNIZING EFFECT OF DIFFERENT DOSES OF *Toxoplasma* RNA

Challenged with 5×10^4 *Toxoplasma* trophozoites at:	Dose of RNA (μg)	Percentage surviving for 20 days (number surviving/total)	*P* value
15 days	200	9 (2/21)	0·65
	50	0 (0/13)	0·40
	Controls	9 (2/23)	—
30 days	200	59 (16/27)	0·02
	50	29 (4/14)	0·63
	Controls	29 (9/31)	—

TABLE 3

PROTECTIVE EFFECT OF *Toxoplasma* RNA INCORPORATED INTO FREUND'S INCOMPLETE ADJUVANT

Immunization	Percentage surviving for 20 days (number surviving/total)	*P* value
RNA (50 μg) + FIA	53 (8/15)	0·007
FIA	7 (1/15)	—
RNA (50 μg)	27 (4/15)	0·16
Controls	0 (0/15)	—

($P = 0{\cdot}02$) was observed only in those mice which had received 200 μg of RNA at 30 days challenge. *Toxoplasma* antibodies were not demonstrable in the serum of mice immunized with RNA.

Representative results of an experiment in which RNA mixed with FIA was used are shown in Table 3. Fifty micrograms of RNA administered with FIA conferred significant protection at 30 days. The same dose without adjuvant did not confer any significant protection.

EFFECT OF MOUSE MACROPHAGE RNA

The result of an experiment in which macrophage RNA and *Toxoplasma* RNA were employed in parallel is shown in Table 4. Two hundred micrograms of RNA extracted from normal macrophages conferred a significant ($P = 0{\cdot}03$) resistance against *Toxoplasma* challenge at 30 days.

EFFECT OF SYNTHETIC POLYRIBONUCLEOTIDES

The results of experiments with polyribonucleotides are shown in Table 5. Poly I:C in the dose employed did not afford protection; poly A:U appeared to give protection but this was not statistically significant ($P = 0{\cdot}08$); poly C afforded a significant protective action ($P = 0{\cdot}04$). When the polynucleotides were mixed with adjuvant, poly A:U conferred significant protection ($P = 0{\cdot}003$), whereas the protection conferred by the other two polynucleotides was not significantly altered.

TABLE 4

PROTECTIVE EFFECT OF *Toxoplasma* RNA AND NORMAL MOUSE MACROPHAGE RNA

Immunization	Dose (μg)	Percentage surviving for 20 days (number surviving/total)	*P* value
Toxoplasma RNA	200	44 (12/27)	0·05
Macrophage RNA	200	58 (11/19)	0·03
Controls	—	27 (8/30)	—

TABLE 5

EFFECT OF SYNTHETIC POLYRIBONUCLEOTIDES IN PROTECTING MICE AGAINST CHALLENGE WITH *Toxoplasma*

Polyribonucleotide	Dose (μg)	FIA	Percentage surviving for 20 days (number surviving/total)	*P* value
Poly I:C	100	No	20 (3/15)	0·29
Poly A:U	100	No	33 (5/15)	0·08
Poly C	100	No	40 (6/15)	0·04
Controls	—	No	7 (1/15)	—
Poly I:C	100	Yes	20 (3/15)	0·11
Poly A:U	100	Yes	47 (7/15)	0·003
Poly C	100	Yes	33 (5/15)	0·02
Controls	—	Yes	0 (0/15)	—

EFFECT OF RNA TREATED WITH PRONASE AND RIBONUCLEASE (RNase)

The results of this experiment are shown in Fig. 2. Seventy-three per cent of the controls, 36 per cent of the mice inoculated with pronase-treated RNA ($P = 0{\cdot}04$), 38 per cent of the mice inoculated with RNA only ($P = 0{\cdot}05$), and 69 per cent of the mice inoculated with RNase-treated RNA ($P = 0{\cdot}5$) died after the challenge with *Toxoplasma*. Although mice inoculated with RNase-treated RNA started dying 7 days after the first deaths had occurred in the controls, the mortality in both groups was the same. Mice injected with RNA only or with pronase-treated RNA had a similar mortality rate and began dying 3–6 days after the earliest deaths had occurred in controls.

In vivo ACTIVATION OF MACROPHAGES BY *Toxoplasma* RNA

Experiments were performed to determine if macrophages from mice previously injected with RNA were activated to resist *Listeria monocytogenes*. Two separate experiments

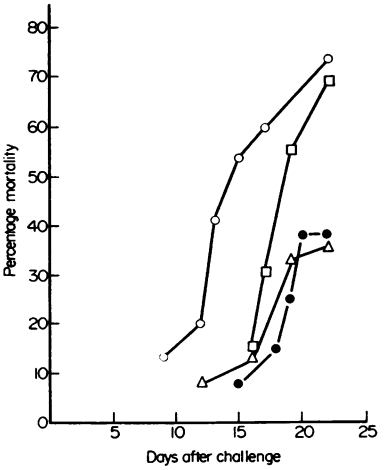

FIG. 2. Mortality in mice immunized with (●) *Toxoplasma* RNA, (□) *Toxoplasma* RNA treated with RNase, and (△) *Toxoplasma* RNA treated with pronase. (○) Controls.

TABLE 6

MACROPHAGE ACTIVATION IN MICE IMMUNIZED WITH *Toxoplasma* RNA

Days after immunization with RNA	Mice	Percentage of macrophages surviving			
		4 hours	*P* value	8 hours	*P* value
15	Injected with 200 μg of *Toxoplasma* RNA	95	0·001	18	0·002
	Chronic *Toxoplasma* infection	83	0·001	28	0·001
	Normal	34	—	4	—
30	Injected with 200 μg of *Toxoplasma* RNA	83	0·02	32	0·8
	Chronic *Toxoplasma* infection	75	0·38	45	0·02
	Normal	69	—	34	—

were performed and the results were essentially the same in each. The result of one experiment is shown in Table 6. Macrophages from mice injected with *Toxoplasma* RNA 15 days earlier were resistant to *Listeria*. This was not true for macrophages obtained from mice which had received *Toxoplasma* RNA 30 days earlier.

DISCUSSION

In previous studies reported from this laboratory, mice treated with vaccines containing whole, killed *Toxoplasma* were significantly protected from a lethal challenge with this

protozoan (Krahenbuhl, Ruskin and Remington, 1972). The result of the present study reveals that fractions from disrupted *Toxoplasma* obtained by various centrifugal forces also can confer resistance in mice to this organism. Of interest was the fact that each of the sediments tested was capable of conferring significant protection in mice to *Toxoplasma* challenge although when equivalent amounts were used the fractions varied in their effectiveness; fraction 1, which contained nuclei and cell wall, was the most potent immunogen. Our findings with respect to *Toxoplasma* are similar to those of Youmans and Youmans (1965) with respect to *Mycobacterium tuberculosis*. Sediments of mechanically ruptured mycobacteria, obtained after centrifugation at 20,360 ***g*** and 56,550 ***g***, were highly immunogenic, whereas the sediment obtained at 144,000 ***g*** had only slight activity in immunization of mice. However, when this latter sediment was mixed with FIA the immunogenic activity was equal to that of the other two sediments. A similar observation was reported by Venneman *et al.* (1970), and Johnson (1972) who noted that ribosomes obtained from *Salmonella typhimurium* conferred protection against salmonellosis when employed without adjuvant.

The recent successful isolation and characterization of *Toxoplasma gondii* RNA (Remington *et al.*, 1970) gave us the opportunity to study its immunizing capacity. When FIA was not used, significant protective effect against lethal challenge was achieved only in mice injected with a dose of 200 μg of *Toxoplasma* RNA and challenged 30 days later. A 50 μg dose was protective only when incorporated into FIA and with the challenge dose given at 30 days. This result differed from that reported by Venneman *et al.* (1970) who demonstrated significant protection against salmonellosis at 15 days with a dose as low as 27 μg of RNA extracted from ribosomes of *S. typhimurium* employed without adjuvant. A significant protective effect was observed when the same dose of RNA was incorporated into FIA and the challenge was given at 30 days but not at 15 days. Thus, the FIA appeared to suppress the early protective effect of the RNA when it was administered alone. In explanation these workers suggested that incorporation of the RNA into adjuvant may suppress either the ingestion of the preparation by macrophages or prevent 'processing' of the antigen by macrophages, thereby delaying the early acquisition by the host of effective anti-*Salmonella* immunity.

In contrast to the antibody response noted in mice injected with the fractions obtained by sedimentation, no humoral response, as detected by the Sabin–Feldman dye test, was demonstrated in the mice injected with *Toxoplasma* RNA. In previous work conducted in this laboratory (C. H. Lai, unpublished results) it was demonstrated that rabbits injected with phenol-extracted RNA also do not form *Toxoplasma* antibodies as measured by the dye test. Whether antibody was responsible for the resistance conferred by the sedimented fractions is unclear. In previous studies we have shown the effectiveness of antibody alone in conferring protection against toxoplasmosis in mice (Krahenbuhl *et al.*, 1972). Although a major role for humoral immunity in toxoplasmosis is unlikely, even a minor role could be operative in this system in which survival is the only parameter measured for resistance and a lethal but not overwhelming challenge is applied. In this context, the interpretation of the data of Collins (1969) on resistance to salmonellosis in mice appears particularly pertinent. It is Collins' contention that antibody alone is protective in the sense that it slows, but does not prevent, the dissemination of the challenge organisms. Thus valuable time is available to the host allowing for development of a 'cellular' immune response to the challenge before being overwhelmed. A similar situation would explain our results in animals immunized with *Toxoplasma* fractions alone. The absence of demonstrable antibody

in the RNA-injected mice suggests the possibility that the observed resistance was non-specific.

Venneman *et al.* (1970), and Winston and Berry (1970a) suggested that ribosomal RNA may act as an adjuvant for an as yet unidentified ribosomal immunogen. In another study Venneman and Berry (1971) observed that immunity induced by their ribosomal vaccine could be passively transferred with peritoneal cells but not with serum from immunized donors. Venneman *et al.* (1970) suggested that the ribosomal RNA or RNA–protein complex may induce the production of an immune effector in mononuclear phagocytes, and Patterson and Youmans (1970) demonstrated that spleen lymphocytes from mice immunized with ribosomal vaccines prepared from *M. tuberculosis* produced a substance which inhibited multiplication of tubercule bacilli in normal mouse peritoneal macrophages. That preparations of *Toxoplasma* RNA can activate macrophages is shown by the results of the present study. Macrophages harvested from the peritoneal cavity of mice injected 15 days earlier with *Toxoplasma* RNA were activated to resist a challenge with *Listeria monocytogenes in vitro*. The studies were performed using macrophages of mice which had been vaccinated with RNA without adjuvant, as we have previously observed that vaccination with complete Freund's adjuvant alone will activate macrophages (Ruskin and Remington, 1971). The fact that macrophages obtained from mice injected 30 days earlier with RNA were not activated to resist bacterial challenge *in vitro* most likely reflects loss of the immunogenic stimulus, probably due to *in vivo* enzymatic degradation of RNA. Since the method employed to demonstrate activation of macrophages is a relatively insensitive one, the protection of the same mice against *Toxoplasma* challenge was possibly due to the presence of a population of activated macrophages not demonstrable by the *in vitro* methods employed in the present study.

The protective effect afforded by *Toxoplasma* RNA in the present study was eliminated by treatment of the RNA with bovine pancreatic ribonuclease but not by pronase. Thompson and Snyder (1971) noted that treatment with ribonuclease destroyed the capacity of a ribosomal preparation to protect mice against *Diplococcus pneumoniae* challenge, whereas treatment with protease decreased the protective effect but did not eliminate it. Youmans and Youmans (1969) reported that in their *M. tuberculosis* model protection was significantly reduced but not destroyed by pancreatic ribonuclease treatment of ribosomes and RNA. In contrast, the protective effect conferred by *S. typhimurium* RNA was not influenced by prior treatment of the RNA with trypsin, deoxyribonuclease, pronase plus ribonuclease, or ribonuclease alone (Venneman *et al.*, 1970).

The ability of phenol-extracted *Toxoplasma* RNA to retain immunogenicity following treatment with pronase and the loss of this immunogenicity following treatment with ribonuclease suggests that the immunizing moiety responsible for the observed protection against *Toxoplasma* was RNA. However, since ribonuclease may have eliminated RNA from a RNA–protein complex which may be resistant to the action of pronase, the possibility that RNA acted as an adjuvant was not excluded. From the results of other workers employing RNA of different species of bacteria it appears that both ribonuclease-resistant and ribonuclease-sensitive preparations have been observed to effectively confer protection in mice (Venneman and Berry, 1971; Venneman and Bigley, 1969; Youmans and Youmans, 1969, 1970).

In order to control further the experiments performed with either the sedimented fractions of disrupted *Toxoplasma* or the *Toxoplasma* RNA, we employed RNA extracted from normal mouse macrophages. When 200 μg of macrophage RNA and *Toxoplasma*

RNA were studied in parallel, both preparations conferred resistance against *Toxoplasma*. Although these results suggest that the protection noted with *Toxoplasma* RNA was due to a non-specific stimulation of the immune system they do not rule out the possibility that the macrophage RNA was a far more potent non-specific stimulator and that at least a portion of the resistance conferred by *Toxoplasma* RNA was specific in nature. Although macrophage RNA can evidently maintain fragments of antigen in antigenic configuration (Askonas and Rhodes, 1965) this could not explain our results obtained with macrophage RNA since the RNA was extracted from macrophages of mice which had not been previously exposed to *Toxoplasma*.

The observation that RNA may non-specifically enhance resistance against *Toxoplasma* suggests that the mechanism of induction of the resistance may be similar to that conferred against *Listeria monocytogenes* in mice inoculated with synthetic polynucleotides or pyran copolymer (Remington and Merigan, 1970). A number of studies have been performed by other investigators to evaluate polynucleotide interferon-inducers in resistance against organisms other than viruses. Youmans and Youmans (1971) employed polyadenylic, polyuridylic and polyadenylic–uridylic and found that these polynucleotides could not replace mycobacterial RNA in inducing protection in mice against virulent *M. tuberculosis*. Herman and Baron (1971) working with *Trypanosoma congolense* were able to demonstrate that poly I:C but not poly A:U conferred resistance in mice against a 'sensitive' variant of the organism. The authors failed to define the mechanism of resistance conferred by poly I:C as being due to interferon and concluded that the protection they observed was possibly due to immunological enhancing capacity of the polynucleotide rather than to their capacity to induce interferon. Although interferon has been shown to protect cells against destruction by *Toxoplasma*, our data suggest a non-specific immunological effect other than interferon since interferon is not found even 1 week after administration of these agents to mice. Merigan, DeClercq, Finkelstein, Clever, Walker and Waddell (1970) found that the highest level of serum interferon in mice injected with pyran copolymer was 24 hours after injection of the copolymer and by day 5 serum interferon was not demonstrable. When poly I:C was injected, the highest level of interferon was also noted at 24 hours and by 48 hours it was no longer demonstrable in the serum. As observed with *Toxoplasma* and *Listeria* (Remington *et al.*, 1970), protection against Mengo virus induced by synthetic anionic polymers lasted longer than interferon could be detected in the injected mice. Although polynucleotides have been shown to confer resistance against a number of bacterial species in addition to *Listeria monocytogenes* (Giron, Schmidt, Ball and Pindak, 1972; Weinstein, Waitz and Came, 1970), only in the *Listeria* model has the resistance been shown to be due, at least in part, to activated macrophages (Remington *et al.*, 1970). In previous studies we noted that the interferon-inducer pyran copolymer also elicited a protective effect in mice challenged with *Toxoplasma* 60 days after the injection of the copolymer (Remington *et al.*, 1970). The protection against *Toxoplasma* conferred by synthetic polynucleotides and pyran also suggests that the resistance elicited by *Toxoplasma* RNA may have been due solely to a non-specific enhancement of immunity. Whereas poly C alone or poly C incorporated into adjuvant conferred resistance *in vivo* against a lethal challenge with *Toxoplasma*, poly A:U conferred resistance only when mixed with adjuvant. The reason for the lack of activity of poly I:C when administered either alone or with adjuvant is unclear. The incorporation of certain polynucleotides into adjuvant could markedly increase their ability to non-specifically enhance resistance against other pathogenic organisms.

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