INFECTION AND IMMUNITY, June 1971, p. 819-824

Vol. 3, No. 6
Printed in U.S.A.

Effect of Interferon and Interferon Inducers on Infections with a Nonviral Intracellular Microorganism, *Rickettsia akari*[1]

J. KAZAR,[2] P. A. KRAUTWURST, AND F. B. GORDON

Department of Microbiology, Naval Medical Research Institute, Bethesda, Maryland 20014

Received for publication 8 February 1971

The effect of mouse interferon (IF) on the multiplication of *Rickettsia akari* in homologous (L-929) cell cultures and the effect of IF inducers on *R. akari* infection in mice were investigated. There was a reduction in the proportion of cells containing rickettsiae in IF-treated cultures and in the yield of rickettsiae from these cultures, as compared with those from infected cultures without IF. Trypsin treatment and heating for 1 hr at 65 C destroyed this antirickettsial activity of the IF preparation, whereas ultracentrifugation (105,000 × *g* for 90 min) and acidification at *p*H 2.0 did not affect it. There was no evidence that mouse IF inactivated *R. akari* directly, nor did it have an inhibitory effect on multiplication of *R. akari* in heterologous chick embryo cell or monkey kidney cell cultures. Susceptibility of *R. akari* to the action of IF was about 16 times less than that of *Chlamydia trachomatis* and 256 times less than the susceptibility of vesicular stomatitis virus. Mice were not protected from infection with *R. akari* by intraperitoneal injection with IF inducers, Newcastle disease virus ($10^{8.3}$ plaque-forming units/0.2 ml) or polyriboinosinic acid-polyribocytidylic acid complex (poly I:C, 200 μg/0.2 ml), within 24 hr before or 24 hr after intraperitoneal challenge. The yields of *R. akari* harvested from the spleens, livers, and peritoneal washings of infected mice treated with IF inducers were similar to those of infected control mice. Titers of IF in peritoneal washings of treated mice, taken 6 hr after administration of Newcastle disease virus or 9 hr after injection of poly I:C, were 1,024 or 320 units/ml, respectively.

After discovery of interferon (IF; reference 5), it was first assumed that this substance inhibits only viral replication. Later, an inhibitory effect of IF on the multiplication of some nonviral intracellular parasites, such as chlamydiae (4, 12, 19) and protozoa (6, 16, 18), was demonstrated as well as the protective effect of IF inducers against bacterial infection (15, 17, 21).

Some attention has also been paid to interference between rickettsiae and viruses and to a relationship between IF and rickettsiae. The ability of rickettsiae grown in cell cultures to interfere with the multiplication of viruses was demonstrated (9, 10; H. R. Tribble, Jr., H. B. Rees, Jr., and H. J. Hearn, Jr., Bacteriol. Proc., p. 98-99, 1965), and evidence has been reported for induction of IF by rickettsiae in cell cultures (3; H. E. Hopps, et al., Bacteriol. Proc., p. 115, 1964) or in vivo (8). Previous infection with influenza virus reduced the mortality of guinea pigs challenged with *Coxiella burneti*, but the mechanism of this protection was not analyzed (7). In spite of the fact that an interference between tick-borne encephalitis virus and *C. burneti* in chick embryo cell cultures was observed, small amounts of chicken or mouse IF (64 units/ml) had no effect on the multiplication of *C. burneti* in homologous cell cultures (11). Finally, an extract of lungs of mice injected intranasally with *Rickettsia prowazeki* was reported to have a moderate protective effect against rickettsial infection of rabbits and guinea pigs (20), but it is not clear whether IF was involved in this protection.

Because the question of the susceptibility of rickettsiae to IF was not answered previously, the purpose of the present study is to determine whether an inhibitory effect of IF on the multipli-

[1] From the Bureau of Medicine and Surgery, Department of the Navy, research task MR011.02-0004.

[2] This research was conducted while the author was pursuing an NRC-Bureau of Medicine and Surgery Postdoctoral Research Associateship; present address is: Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia.

cation of rickettsiae in cell cultures can be detected and whether IF inducers will exert a protective effect on rickettsial infection in vivo.

MATERIALS AND METHODS

Cell cultures and media. Mouse fibroblast cell (L-929) cultures, rhesus monkey kidney cell (LLC-MK2) cultures, and chick embryo cell (CE) cultures were grown in 32-oz (ca. 900 ml) prescription bottles in Eagle's minimal essential medium (MEM) containing 1% glutamate and 10% inactivated calf serum (ICS). For McCoy cell cultures, MEM enriched with 1% glutamate and 10% inactivated horse serum was used. The same media containing 2% ICS only were used for maintaining the cell cultures and for dilution of inocula. No antibiotics were used, except in the titration of IF when 100 units of penicillin per ml and 100 μg of streptomycin per ml were added.

Microorganisms. *R. akari* strain MK, obtained from B. L. Elisberg of Walter Reed Army Institute of Research, Washington, D.C., and *Chlamydia trachomatis* strain MRC-1/G, characterized elsewhere (2), were grown in the yolk sac of chick embryos, from which 50% suspensions were prepared in sucrose-phosphate buffer solution (23) and stored at −70 C. For some experiments, rickettsial suspensions were partially purified and concentrated by a method described previously (23). Stock preparations were assayed in 6-day-old chick embryos, and the 50% egg infective dose (EID_{50}) per ml was determined by the Reed-Muench formula. Harvests of *R. akari* from cell cultures or from mouse viscera were titrated in gamma-irradiated (5,000 r) McCoy cell cultures grown in flat-bottomed tubes on round cover slips, using the methods employed in this laboratory for chlamydiae (1). A 1-ml amount of each 10-fold dilution of disrupted L cells or of 20% mouse spleen and liver suspensions in peritoneal washings was added to each of four tubes which were then centrifuged horizontally for 1 hr at 1,600 × *g*. After 6 days of incubation at 32 C, cells were fixed with methanol and the cover slips were removed and stained by Giemsa-Romanowski. Titers were expressed in 50% tissue culture infective doses ($TCID_{50}$) by using the presence of rickettsiae, observed microscopically, to determine the end point.

C. trachomatis seed was titrated by using the same method of culture (1), and the titers were expressed as inclusion-forming units (IFU)/ml derived from microscopic counts of inclusions.

Newcastle disease virus (NDV) strain B1, from allantoic fluid of chick embryos, was titrated in CE cell cultures by routine plaque technique. Encephalomyocarditis (EMC) virus strain r^+, propagated in L-cell cultures, and vesicular stomatitis virus (VSV) strain Indiana, propagated in CE cell cultures, were assayed for $TCID_{50}$/ml in L-cell cultures. All viruses were kindly supplied by S. Baron from the National Institutes of Health, Bethesda, Md.

Production of IF. Fifteen-milliliter amounts of allantoic fluid containing $10^{8.3}$ plaque-forming units (PFU)/ml of NDV were exposed in petri dishes to a 15-w ultraviolet lamp at a distance of 7 inches (17.8 cm) for 20 sec. The plates were rocked mechanically at the rate of 90 times per min. Monolayers of L-cell cultures grown in 32-oz prescription bottles were exposed to 10 ml of ultraviolet-treated NDV preparation by using a dose such that the multiplicity of infection before virus inactivation would have been 30 PFU/cell. After 90 min of incubation at 37 C, NDV was removed and cell cultures were washed with MEM and refed with MEM without serum and antibiotics. Medium harvested after 20 to 24 hr of incubation at 37 C was checked for sterility in blood-agar plates and thioglycolate broth and was centrifuged at 105,000 × *g* for 90 min; the supernatant was tested for IF activity. Those noncontaminated preparations which possessed at least 320 units of IF activity per ml were collected, concentrated about 40-fold by vacuum pressure dialysis, and subsequently dialyzed against 0.01 M sodium acetate buffer at *p*H 4.5; the resulting precipitates were removed by centrifugation. The supernatants, after testing again for bacterial sterility and for IF activity, were kept at −20 C. In one experiment, we used a similarly prepared L-cell IF preparation, further purified by chromatography on carboxymethyl Sephadex C-25 and by electrophoresis in polyacrylamide gels and containing $\cong 10^6$ IF units per mg of protein. This preparation was kindly supplied by D. Stanček, Children's Hospital of Philadelphia. The methods for its purification and characterization were described elsewhere (14). In some experiments, IF was induced in female, 25-g NMRI strain mice, by intravenous injection with $10^{8.3}$ PFU per 0.2 ml of NDV. Blood was collected from the sinus orbitalis 8 to 10 hr after NDV administration, and serum was separated by low-speed centrifugation. After ultracentrifugation at 105,000 × *g* for 90 min, the supernatant was acidified with 2 N HCl at *p*H 2.0 and, after 24 hr, was readjusted to *p*H 7.0 with 2 N NaOH and tested for sterility and IF activity.

Titration of IF. Monolayers of L cells in tube cultures were exposed to 1-ml portions of serial twofold dilutions of IF preparations. After overnight incubation at 37 C, the IF-containing medium was removed and 1-ml amounts of VSV or EMC virus were added containing 3×10^2 to 10^3 $TCID_{50}$. The titer of IF (units per milliliter) was expressed as the reciprocal of the highest dilution that completely inhibited the cytopathic effects of VSV or EMC virus at the time when control cultures were totally destroyed by the virus. In all experiments, control material consisted of medium harvested from L-cell cultures that had been exposed to uninfected allantoic fluid or of serum of mice injected intravenously with the same.

Evaluation of antirickettsial effect of IF in cell cultures. Monolayers of L-cell cultures grown in 2-oz (ca. 60 ml) plastic flasks (Falcon) were exposed to 5 ml of IF preparations or to the same amount of control material. After overnight incubation at 37 C, cultures were washed with MEM and infected with 1 ml of the dilution of *R. akari* in maintenance medium to provide a multiplicity of infection (MOI) of approximately 5 EID_{50} per cell. After 2 hr of adsorption at 37 C, the inoculum was removed and the cells were washed twice with MEM and refed with 5 ml of the maintenance medium. Infected cell cultures were incubated at 32 C for 3, 4, or 6 days. At these times, the medium was removed and the cells were suspended by exposure to

1 ml of 0.25% trypsin, centrifuged at 500 rev/min for 10 min, washed in MEM, and, after resuspension in 2 ml of MEM per flask, counted and diluted to a concentration of 10^6 cells per ml. In some experiments, one part of the cell suspension was frozen and thawed three times and kept at −70 C until titration of the yield of *R. akari*. From another portion of the suspension, smears were made, dried in air, and stained by Gimenez. The number of cells containing clearly visible rickettsiae either in cytoplasm or in nucleus in a total of 200 was estimated.

RESULTS

Inhibitory effect of mouse IF on R. akari multiplication in homologous (L-929) cell cultures. The data of Table 1 indicate that the proportion of cells containing rickettsiae 3 or 6 days postinfection was reduced by approximately 60% in cell cultures treated with either mouse serum or L-cell IF. The yields of *R. akari* harvested from IF-treated cultures were decreased 0.5 to 1.2 log units as compared with those from the controls. Control preparations exerted no significant effect on the proportion of infected cells and on the yield of *R. akari*.

Similar results were obtained when L-cell cultures grown in flat-bottomed tubes on round cover slips were exposed to 1 ml of L-cell IF preparation containing 800 IF units and challenged with a partially purified and concentrated pool of *R. akari* at an MOI of about 0.1 EID_{50} per cell, by using centrifugation of inocula into the monolayers. In control cultures, the mean proportion (from triplicate tubes) of the cells containing rickettsiae 4 days postinfection was 79% and the yield of *R. akari* (harvested from 10 tubes) per 10^6 cells was $10^{4.0}$ $TCID_{50}$. In IF-treated cultures, the figures were 17% and $10^{3.0}$, respectively.

TABLE 1. *Inhibitory effect of mouse interferon (IF) preparations on Rickettsia akari multiplication in homologous (L-929) cell cultures*

Treatment of L-cell cultures before infection with *R. akari*[a]	Proportion (%) of cells containing rickettsiae[b]		Infectious titer of harvest of *R. akari* per 10^6 cells	
	3 days[c]	6 days	3 days	6 days
None	23	89	$10^{3.0}$	$10^{4.5}$
L-cell control medium	24	91	$10^{3.0}$	$10^{4.5}$
Mouse serum control	19	82	Not tested	$10^{4.2}$
L-cell IF (256 units/ml)	11	31	$10^{2.5}$	$10^{3.5}$
Mouse serum IF (256 units/ml)	7	28	Not tested	$10^{3.0}$

[a] Triplicate flasks were treated with 5 ml of IF-containing or control preparations.

[b] Two hundred cells from pools of cells from three flasks were examined.

[c] Postinfection.

TABLE 2. *Lack of direct inactivation of Rickettsia akari by interferon (IF) preparations*

Mixture titrated	Titer of *R. akari* ($TCID_{50}$/ml)
R. akari[a] undiluted plus control L-cell medium	$10^{5.0}$
R. akari[a] undiluted plus L-cell IF (256 units/ml)	$10^{5.0}$
R. akari[b] diluted 1:10 plus control mouse serum	$10^{5.5}$
R. akari[b] diluted 1:10 plus mouse serum IF (2,048 units/ml)	$10^{5.3}$

[a] Crude pool of *R. akari*.

[b] Partially purified and concentrated pool of *R. akari*.

Lack of direct inactivation of R. akari by IF preparation. Mixtures of equal parts of *R. akari* suspension and L-cell culture IF that contained 256 units per ml or control L-cell culture medium were incubated for 1 hr at 37 C and titrated in gamma-irradiated McCoy cell cultures as described above. In another experiment performed in the same way, mixtures of 1:10 dilution of crude *R. akari* pool and mouse serum IF containing 2,048 IF units, or control mouse serum diluted equally, were titrated. No significant difference in the titers of rickettsiae exposed to IF or control preparations (Table 2) indicates that IF does not inactivate *R. akari* directly.

Characteristics of interferon preparation used for inhibition of R. akari multiplication. Different portions of crude IF (320 units/ml) prepared in L cells were subjected to the following procedures: centrifugation (90 min at 105,000 × *g*), acidification (to *p*H 2.0 with 2 N HCl for 24 hr and then neutralized to *p*H 7.0 with 2 N NaOH), heating at 37 C or at 65 C for 1 hr, trypsinization (1 hr at 37 C in the presence of 0.25% trypsin after which soy bean inhibitor was added), purification, and chromatography, as described above. L-cell cultures were exposed overnight to the treated preparations, as well as to controls of the crude IF and medium from control L-cell cultures. Similarly, control medium and L-cell IF were used for treatment of chicken (CE) and monkey (LLC-MK2) cells. All tubes were then inoculated with *R. akari*, and 4 days later the proportion of cells containing rickettsiae was estimated (Table 3). In parallel, the IF preparations were tested for antiviral activity against 300 $TCID_{50}$ of VSV in L-cell and chick embryo cultures.

TABLE 3. *Characterization of interferon (IF) preparation used for inhibition of Rickettsia akari multiplication*

Cell cultures	Prepn added[a]	Titer of IF prepn (units/ml) against 300 $TCID_{50}$ of VSV	Proportion (%) ± SE of cells containing rickettsiae 4 days postinfection
Mouse	Control L-cell medium	0[b]	60.6 ± 2.4[c]
	L-cell IF		
	Crude	1,600	16.6
	Centrifuged at 105,000 × *g* for 90 min	1,600	18.6 ± 1.6
	Treated at *p*H 2 for 24 hr	1,280	22.0
	Heated 37 C for 1 hr	1,280	27.0
	Heated 65 C for 1 hr	0	56.3
	Treated with 0.25% trypsin for 1 hr at 37 C	0	57.3 ± 1.7
	Purified, chromatographed	6,400	10.3
Chicken	Control L-cell medium	0	45.0
	Crude L-cell IF	0	43.6
Monkey	Control L-cell medium	Not tested	75.3
	Crude L-cell IF	Not tested	74.3

[a] A 5-ml amount of 1:5 dilution was added to cell cultures and was incubated overnight before inoculation with *R. akari*.

[b] No inhibition of vesicular stomatitis virus (VSV) cytopathic effect in 1:5 dilution of preparation used.

[c] Values derived from examination of 200 cells from each of three replicate cultures. SE, standard error.

Heat (1 hr at 65 C) or trypsin completely destroyed both antiviral and antirickettsial activity of the tested material, whereas ultracentrifugation and acidification had no effect. The same preparations inhibited both VSV and *R. akari* multiplication in homologous L-cell cultures, but their multiplication in heterologous cell cultures was not affected (Table 3).

Comparison of the susceptibility of R. akari, C. trachomatis, and VSV to mouse interferon in homologous (L-929) cell cultures. L-cell cultures were exposed to 5 ml of fourfold dilutions of mouse serum IF, of which the original titer was 20,000 units/ml when tested against 300 $TCID_{50}$ of EMC virus in L cells. After overnight incubation, the cell cultures were infected (1.0 ml) in duplicate with 40 PFU of VSV, or with *C. trachomatis* (MOI = 5 IFU per cell), or with *R. akari* (MOI = 5 EID_{50} per cell). The adsorption period for VSV was 1 hr, and that for chlamydiae and rickettsiae was 2 hr, at 37 C. L-cell cultures infected with the virus were incubated for 2 days at 37 C before calculation of plaque number, those infected with chlamydiae for 2 days at 35 C, and those with rickettsiae for 4 days at 32 C, before estimation of the proportion of infected cells.

Results in Table 4 show different susceptibilities of the three agents to the inhibitory action of IF. Comparison was made by noting the lowest IF titer in each case which caused definite reduction in growth. These were 1, 16, and 256 units, respectively, for VSV, *C. trachomatis*, and *R. akari*. Thus, when tested in this manner, *C. trachomatis* (MRC-1/G) is about 16 times less susceptible to IF than is VSV, and *R. akari* is about 16 times less susceptible than is *C. trachomatis*.

Effect of IF inducers on intraperitoneal infection of mice with R. akari. Female white mice (NMRI strain) weighing 15 g, 20 mice per group, were injected intraperitoneally with 0.2 ml ($10^{8.3}$ PFU) of NDV or with 0.2 ml (200 μg) of polyriboinosinic acid-polyribocytidylic acid complex (poly I:C; Microbiological Associates, Inc., Bethesda Md.) at intervals of 6 or 9 and 24 hr before challenge intraperitoneally with $10^{6.5}$ EID_{50} per 0.4 ml of *R. akari* (Table 5). At the time of challenge, other mice of the same groups were sacrificed and their peritoneal cavities were washed with MEM. Washings from three mice of each group were pooled (6 ml), centrifuged lightly, and tested for IF activity. The challenged mice were observed during a period of 10 days and deaths were tabulated. At 3 and 6 days after infection, three mice from each group were sacrificed, their peritoneal cavities were washed with 2 ml of MEM, and spleens and livers were harvested. Twenty per cent suspensions of these viscera in peritoneal washings and additional MEM were titrated after storage (−70 C) for infectious *R. akari* in irradiated McCoy cell cultures.

No significant difference in the mean survival time of mice injected with IF inducers as com-

TABLE 4. *Comparison of the susceptibility of Rickettsia akari, Chlamydia trachomatis, and vesicular stomatitis virus (VSV) to mouse interferon (IF) in homologous (L-929) cell cultures*

Treatment of L-cell cultures before infection[a]	IF titer (units/ml)[b]	No. of VSV plaques per flask	Proportion (%) of 200 cells containing	
			C. trachomatis (2 days postinfection)	*R. akari* (4 days postinfection)
None	<10	39, 41	78, 82	72, 74
Mouse serum control (1:10)	<10	32, 35	66, 68	63, 66
Mouse serum interferon				
1:5	4,095	Not tested	Not tested	3, 6
1:20	1,024	Not tested	1, 2	7, 9
1:80	256	0, 0	2, 3	11, 14
1:320	64	0, 0	7, 11	54, 57
1:1,280	16	0, 0	17, 23	69, 71
1:5,120	4	0, 0	53, 61	73, 76
1:20,480	1	2, 5	73, 80	Not tested
1:80,000	0	31, 33	Not tested	Not tested

[a] Overnight treatment at 37 C with 5 ml per flask of dilution of mouse serum previously subjected to centrifugation at 105,000 × *g* for 90 min and acidification at *p*H 2 for 24 hr.

[b] Titer of IF in units/ml derived from previous test with 300 $TCID_{50}$ of EMC virus.

TABLE 5. *Effect of interferon (IF) inducers on intraperitoneal infection of mice with Rickettsia akari*

IF-inducer and interval before challenge[a]	Titer of IF in peritoneal washings[b] (units/ml)	Mean survival time of group of 14 mice (days)	Infectious titer of viscera post-infection[c]	
			3 days	6 days
None	<10	8.2	$10^{2.8}$	$10^{5.5}$
Poly I:poly C (9 hr)	320	8.0	$10^{2.8}$	$10^{5.5}$
Poly I:poly C (24 hr)	160	8.5	$10^{2.5}$	$10^{5.3}$
NDV (6 hr)	1,280	8.0	$10^{2.8}$	$10^{5.5}$
NDV (24 hr)	40	7.4	$10^{3.1}$	$10^{5.8}$

[a] Challenge with 200 μg per 0.2 ml of poly I:C or $10^{8.3}$ PFU per 0.2 ml of Newcastle disease virus (NDV) injected intraperitoneally before challenge with $10^{6.5}$ EID_{50} per 0.4 ml of *R. akari*; 20 mice per group.

[b] Titer of pooled peritoneal washings of three mice collected at the time of rickettsial inoculation.

[c] Titers of *R. akari* from 20% suspensions of spleens and livers pooled in peritoneal washings; three mice in each group.

pared with control animals was found (Table 5). The yields of rickettsiae from pooled viscera and peritoneal washings of treated mice were also not affected. Peritoneal washings taken at the time of challenge from mice that had received an inducer 6 or 9 hr previously possessed levels of IF equal to those that inhibit *R. akari* in cell cultures. At 24 hr, the IF level in peritoneal washings had dropped below the critical level for rickettsial inhibition in vitro, and, in additional assays performed at 48 hr (not recorded in Table 5), no IF was detected.

In another experiment, mice were injected intraperitoneally with NDV ($10^{8.3}$ PFU in 0.2 ml) or poly I:C (200 μg in 0.2 ml) 24 hr after intraperitoneal infection with $10^{6.5}$ EID_{50} per 0.4 ml of *R. akari*. Again, no difference was found either in the mean survival time or in the presence of rickettsiae stained by Gimenez in viscera and in cells lining the peritoneal cavity between groups of treated and control animals.

DISCUSSION

The preparation derived from L-cell cultures, demonstrated in this study to have antirickettsial activity, possessed all of the essential characteristics of IF, namely, host cell specificity, viral nonspecificity, nonsedimentability, *p*H stability, and trypsin susceptibility. These results allow us to conclude that IF was responsible for the observed inhibition of *R. akari* multiplication. Lack of direct inactivation of *R. akari* by IF preparations and host cell specificity for antirickettsial activity indicate that this effect was mediated by the host cells.

The inhibition by IF of rickettsial multiplication is not as pronounced as that of a virus. Not only were higher doses of IF necessary for reduction of *R. akari* multiplication, at least 256 units/ml as compared to 1 unit/ml for VSV, but as much as 4,096 units/ml did not inhibit rickettsial multiplication completely. When the susceptibilities of virus, chlamydiae, and rickettsiae to IF were compared, *C. trachomatis* was about 16 times less susceptible than VSV, which is in agreement with a previous finding (4), and *R. akari* is even

less susceptible to IF than *C. trachomatis*. It is possible that the less marked susceptibility to IF of nonviral intracellular microorganisms, such as chlamydiae and rickettsiae, can be explained by their lesser dependency on host cells. Rickettsiae and chlamydiae possess some host cell-independent but different enzymatic activities. The reactions carried out by chlamydiae often require cofactors and do not result in net synthesis of adenosine triphosphate. Rickettsiae, on the other hand, can generate at least some of their own adenosine triphosphate (22). It is impossible to say whether these differences between chlamydiae and rickettsiae might be responsible for their different susceptibility to IF because there are no data available on the mechanism of IF inhibitory activity on nonviral intracellular microorganisms.

Another question is the importance of IF in rickettsial infection in vivo. IF was demonstrated in the serum of mice intravenously injected with *C. burneti* and *R. prowazeki* (8), but no IF was detected in peritoneal washings, viscera, and serum of mice up to 8 days postintraperitoneal infection with these rickettsiae (J. Kazar, *unpublished data*).

Failure of IF inducers to protect mice against rickettsial infection in the present investigation can be explained by our observation (Table 4) of a relative insensitivity of rickettsiae to the action of IF in cell cultures, but a possible role, under other circumstances, of IF or IF inducers in rickettsial infection of whole animals is not excluded. A protocol in which a significant level of IF was sustained for a longer period, or in which the interval between injection of IF inducer and challenge was different, might have given evidence of protection. Experiments of the latter type are complicated, however, by the possiblity of protection of animals, after injections of IF inducers, without the mediation of IF, as suggested in experiments with bacterial (21) and chlamydial (13) infection.

LITERATURE CITED

1. Gordon, F. B., and A. L. Quan. 1965. Isolation of the trachoma agent in cell culture. Proc. Soc. Exp. Biol. Med. **118**: 354–359.
2. Gordon, F. B., and A. L. Quan. 1965. Occurrence of glycogen in inclusions of the psittacosis-lymphogranuloma venereum-trachoma agents. J. Infec. Dis. **115**:186–196.
3. Hahon, N., and E. H. Kozikowski. 1968. Induction of interferon by *Coxiella burneti* in cell cultures. J. Gen. Virol. **3**: 125–127.
4. Hanna, L., T. C. Merigan, and E. Jawetz. 1966. Inhibition of TRIC agents by virus-induced interferon. Proc. Soc. Exp. Biol. Med. **112**:417–421.
5. Isaacs, A., and J. Lindenmann. 1957. Virus interference. I. The interferon. Proc. Royal Soc. Ser. B **147**:258–267.
6. Jahiel, R. I., J. Vilcek, R. S. Nussenzweig, and J. Vandenberg. 1968. Interferon inducers protect mice against *Plasmodium berghei* malaria. Science **161**:802–804.
7. Janssen, R. J., P. J. Gerone, and W. A. Chappell. 1965. Interference with *Coxiella burnetii* infections of guinea pigs by influenza A virus. J. Bacteriol. **89**:1628.
8. Kazar, J. 1966. Interferon-like inhibitor in mice sera induced by rickettsiae. Acta Virol. **10**:277.
9. Kazar, J. 1969. Interference between *Coxiella burneti* and cytopathic viruses in chick embryo cell cultures. I. Establishment of the interference. Acta Virol. **13**:124–134.
10. Kazar, J. 1969. Interference between *Coxiella burneti* and cytopathic viruses in chick embryo cell cultures. II. An attempt to elucidate the mechanism of the interference. Acta Virol. **13**:216–225.
11. Kazar, J. 1969. Multiplication of *Coxiella burneti* in virus-infected and interferon-treated cell cultures. Acta Virol. **13**: 346–348.
12. Mordhorst, C. H., V. Reinicke, and E. Schonne. 1967. In ovo inhibition by concentrated chick interferon of the growth of TRIC agents. Amer. J. Ophthalmol. **63**:1107–1109.
13. Oh, J. O., H. B. Ossler, and J. Schachter. 1970. Protective effect of a synthetic polynucleotide complex (poly I:C) on ocular lesions produced by trachoma agent in rabbits. Infec. Immun. **1**:566–573.
14. Paucker, K., B. J. Berman, R. R. Golgher, and D. Stanček. 1970. Purification, characterization, and attempts at isotopic labeling of mouse interferon. J. Virol. **5**:145–152.
15. Pindak, F. F. 1970. Protection of mice against bacterial infection by interferon inducers. Infec. Immun. **1**:271–273.
16. Remington, J. S., and T. C. Merigan. 1968. Interferon: protection of cells infected with intracellular protozoan (*Toxoplasma gondii*). Science **161**:804–806.
17. Remington, J. S., and T. C. Merigan. 1970. Synthetic polyanions protect mice against intracellular bacterial infection. Nature (London) **226**:361–363.
18. Schultz, W. W., K. Huang, and F. B. Gordon. 1968. Role of interferon in experimental malaria. Nature (London) **220**: 709–710.
19. Sueltenfuss, E. A., and M. Pollard. 1963. Cytochemical assay of interferon produced by duck hepatitis virus. Science **139**: 595–596.
20. Wei, W.-P., C. W. An, and L. H. Chin. 1966. Some observations on the production of rickettsial interferon from mouse lung. Acta Microbiol. Sinica **12**:29–33.
21. Weinstein, M. J., J. A. Waitz, and P. E. Came. 1970. Induction of resistance to bacterial infections of mice with poly I·poly C. Nature (London) **226**:170.
22. Weiss, E. 1968. Comparative metabolism of rickettsiae and other host dependent bacteria. Zentralbl. Bakteriol. Parasitenk. Infek. Hyg. **206**:292–298.
23. Weiss, E., H. B. Rees, Jr., and J. R. Hayes. 1967. Metabolic activity of purified suspensions of *Rickettsia rickettsi*. Nature (London) **213**:1020–1022.