POSSIBLE ROLE OF INTERFERON IN CONTROL OF RESPIRATORY INFECTIONS IN RECRUITS

Michael W. Rytel

Medical College of Wisconsin

Prepared for:

Office of Naval Research

31 August 1974

UNCLASSIFIED

Security Classification

AD 784 431

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)

Medical College of Wisconsin
Milwaukee, Wisconsin

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3. REPORT TITLE

Possible Role of Interferon in Control of Respiratory Infections in Naval Recruits

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final report

5. AUTHOR(S) (First name, middle initial, last name)

Michael W. Rytel, M.D.

6. REPORT DATE

August 31, 1974

7a. TOTAL NO. OF PAGES

15

7b. NO. OF REFS

8a. CONTRACT OR GRANT NO.

N00014-67-A-0424

b. PROJECT NO. 71

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

Work unit NR136-941

10. DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY

Bureau of Medicine and Surgery
The Office of Naval Research
Navy Department, Washington, DC

13. ABSTRACT

The purpose of this study was to extend the spectrum of vaccines which are known to be interferon inducers in man. Five subjects, two of whom had low or undetectable polio type 2 neutralizing antibody levels were given the standard type 2 vaccine by mouth. Even though the two individuals with low initial titers experienced a fourfold or greater neutralizing antibody rise and one of them shed the virus in his stool, neither they nor the remaining three volunteers developed detectable levels of interferon in their sera. Fifteen subjects were given approximately $10^{7.5}$ $TCID_{50}$ of influenza A/England/42/72 by nasal drops. Specimens consisting of sera and nasal washings were obtained at closely timed intervals for 23 days, starting with day 3 following immunization. The interferon response occurred in 33.3% of the subjects with fourfold or greater HI antibody response. No interferon was detected in nasal washings. It is concluded that polio is not an active interferon inducer in man. Live attenuated influenza vaccine does produce an interferon response in subjects with initial low serum antibody titers. This latter finding also suggests that the attenuation of the vaccine strain is not due to its ability to produce interferon.

An additional aspect of this study included evaluation of the possible induction of interferon antagonists by viral agents. The results indicate that noninfected mice appear to possess an antagonist of interferon action in their sera and tissue extracts. During infection with coxsackie B3 when interferon is stimulated, the antagonist effect appears to be no higher than in normal mice, and its effect is blunted by the presence of the induced interferon in the samples being assayed for antagonist.

i.

15

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

5

Unclassified

Security Classification

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Interferon Induction						
Vaccines						
Interferon Antagonist						

ia

INDUCTION OF INTERFERON IN MAN BY VACCINES

Several vaccines have been reported in the literature as having the potential of interferon induction (1-4). In view of the fact that no other safe and effective interferon inducers are currently available, these vaccines remain the only readily available agents which could be employed for possible prophylactic or therapeutic interferon induction in man. The purpose of this study was to attempt to extend the spectrum of vaccines with known interferon inducing potential. Two vaccines were evaluated. One was a commercially available live, oral poliomyelitis vaccine, type 2, and the other a new live, attenuated influenza A/England/42/72 (H3N2) vaccine which is currently undergoing field trials.

Respiratory viral infections constitute a major health problem in military recruits (5). The spectrum of the currently available vaccines effective against respiratory infections is narrow. Particularly insofar as rhinoviral infections are concerned, the prospects for the development of effective specific vaccines are bleak owing to the great number of viral serotypes. In view of this, interferon is potentially of great importance because of its broad spectrum of activity and its demonstrated efficacy in respiratory infections.

Respiratory viral infections occur in the military recruits newly arrived in training camps in a very predictable manner, with the highest incidence occurring during the first four weeks of training (6). This is also the time when recruits receive most of their immunizations; however, the schedule of these is, of course, not based on the consideration of their possible interferon inducing potential. It was our hope that once the vaccines capable of interferon induction were identified, they could be administered to the recruits on a schedule (perhaps at weekly intervals) which could result in sustained interferonemia and hopefully, protection against respiratory infections.

Materials and Methods. Poliomyelitis Vaccine. Poliovirus vaccine, live, oral, type 2, Sabin strain, Wyeth, Lot No. 17702 was given by mouth in a dose of approximately $10^{4.7}$ $TCID_{50}$. The type 2 vaccine was chosen because of the three monovalent types available, it has been associated with the fewest complications (product information, Wyeth). Blood samples were obtained on days 0, 2, 5, 10, 15, 20, and 25 from five volunteers. Rectal swabs were also obtained on day 5, as in previous studies this had been determined to be the time of maximal fecal excretion. Neutralizing antibody titers were performed in the standard manner employing 100 $TCID_{50}$ of the type 2 vaccine strain of poliovirus. Serial serum dilutions in 2% E-MEM were incubated with the viral inoculum for one hour at room temperature and then 0.2 ml of the mixture was added to WI-38 tubes (Microbiological Associates [MBA], Bethesda, Md.). The end point was taken as the highest dilution giving 50% suppression of the CPE. Viral isolation was carried out in both primary rhesus monkey kidney cells and in WI-38 cells (MBA).

Influenza Vaccine. "Alice strain" of A/England/42/72 (H3N2) vaccine (Smith Kline and French Co., Philadelphia, Pa.) was given in a dose of approximately $10^{7.5}$ $TCID_{50}$ by nasal drops on day 0 and 14 of the study. Blood was obtained for interferon and HI antibody determinations on days 0, 3, 7, 16, 23 and 30. Nasal washes were obtained on the same days utilizing Ringer's lactate solution. Viral isolation was performed on nasal washings in primary rhesus monkey kidney tubes (MBA) which were incubated on a roller drum at 33°C and 12 rev/hour. Hemadsorption was performed with 0.5% guinea pig erythrocytes following incubation for 7 and 14 days and after one blind passage. Hemagglutination inhibition antibody titers were done according to standard techniques, employing four hemagglutinating units of influenza A/England/42/72 (H3N2) (Smith Kline and French). All sera were treated with receptor destroying enzyme (RDE) of Vibrio cholerae (MBA). Following overnight incubation at 37°C, RDE and complement were inactivated by 2.5% sodium citrate and heating at 56°C for 30 minutes. Microtiter "U"-plates were used in the procedure (MBA).

Interferon Titration. Petri dishes (60 x 15 mm) (Falcon Plastics) were seeded with 2 x 10^6 human foreskin fibroblasts per plate. They were then incubated at 37°C in an atmosphere of about 5% CO_2 for two to four days until completely sheeted out. The growth medium was removed and the monolayers washed one time with phosphate buffered saline (PBS). Twofold dilutions (1:10 to 1:10,240) of the samples assayed for interferon were made in maintenance media (E-MEM with 2% FCS, 100 units of penicillin-G, and 10 mcg of gentamicin/ml). Two ml of each dilution was added in duplicate, and the plates were then incubated for 6 hr at 37°C and 5% CO_2. Afterwards, the samples were aspirated and the monolayers washed two times with PBS. Vesicular stomatitis virus (VSV-Indiana Strain), appropriately diluted to give 100 PFU per plate, was added in a volume of 0.2 ml to each plate. After 30 minutes incubation at 37°C, the cell sheet was overlayed with 5 ml of nutrient agar. This is prepared in the following ratios per 100 ml of final solution: 50 ml of 2% Ionagar (Oxoid Division, Consolidated Laboratories, Chicago Heights, Ill.), 50 ml of 2 x 199 containing 4 ml MEM (50X) amino acids, 2 ml (100X) vitamins. Twenty-four hours later, a second agar overlay was added, this time containing neutral red in a final concentration of 0.005%. After an additional 24 hr of incubation, the plaques were counted. The interferon titer (in "units") was expressed as the reciprocal of the highest dilution suppressing plaque formation by 50% as compared to virus controls. Employing this assay system, British human research interferon standard A 69/19 was found to have a titer of 5990 units.

Results. Results of the attempted induction of interferon with the polio vaccine are summarized in Table 1. Even though one subject had undetectable neutralizing antibody titer and experienced a very satisfactory rise in neutralizing antibodies, and another subject who had been immunized ten years previously also exhibited a significant antibody rise, neither they nor any of the other volunteers showed interferon response. The sera were obtained on numerous occasions following attempted induction. The times were chosen based on the reports in the Russian literature on interferon induction following oral poliovirus vaccine (7).

With the live attenuated influenza virus vaccine, interferon was detected in three of 15 volunteers (20%); all three of these exhibited a fourfold HI antibody response (Table 2). The overall interferon response in the nine subjects who showed an equal to or greater than fourfold rise in HI antibodies was 33.3%. No interferon was detected, however, in the sera or the nasal washings of a subject (B.T.) who had shed the virus 3 days following the first dose of the vaccine, nor in another subject (L.A.) from whom virus was also recovered. No interferon was detected in the nasal washings of any of the other subjects in the study, nor in the sera of subjects other than those alluded to above.

Discussion. Interferon induction has been reported following administration of a number of currently available vaccines (1-5, 7, 8). These are summarized in Table 3. In general, most of the vaccines which have been found to be good interferon inducers have been comprised of myxoviruses or paramyxoviruses such as measles, mumps and rubella. It is well recognized that interferon response is more marked following induction by myxo than many other viruses(9). For example, in our previous studies, NDV produced high interferon serum titers in mice, whereas coxsackievirus B_3 infection was associated with but a meager interferon response (10). It is thus perhaps not surprising that in this study, interferon was detected in 33.3% of subjects who developed a significant HI antibody response following intranasal administration of live attenuated influenza A/England/42/72 (H3N2) vaccine, but in none of the subjects following poliovirus administration. The latter finding is at variance with reports in the Russian literature where interferon response had been reported following both polio type 2 and "live enteroviral vaccines" comprising ECHO viruses (7, 8). The explanation for this is not readily apparent, but is unlikely to be due to the insensitivity of our interferon assay system because the British human interferon standard 69/19 which contains approximately 5000 units was found to have 5990 units by our assay.

The interferon response following immunization with our strain of influenza virus occurred in fewer subjects, and the levels were lower than in a study reported by Jao, et al. in which Bethesda 10/63 strain of influenza A_2 was employed (11). Their strain, however, was less attenuated than ours. In a recent report by Murphy et al. where interferon response was studied following administration of two influenza virus strains, one a wild type influenza/A/Bethesda/1968 (H3N2) and the other, a temperature-sensitive recombinant virus, influenza A (H3N2)-ts-1-[E], a consistantly lower and less prolonged interferon response was found with the attenuated strain (12). Interferon was detected in 47% of the subjects infected with the latter strain, which is not significantly different from our figure of 33.3%. The peak of interferonemia occurred on day 2 with the attenuated, and day 3 with the wild strain. We have not commenced our sampling until day 3 following immunization (based on the previously reported results of Jao, et al. (11) who found maximal interferon response on day 4), and thus, we may have missed the time of peak response. Our data also suggests that the attenuation of the vaccine employed in this study was not due to its high interferon-inducing potential.

What then are the prospects for the possible use of vaccines as interferon inducers in man? Their role will probably be quite limited since the interferon response decreases following their repeat administration. Some vaccines (influenza A_2) appear to lose their interferon-inducing potential with attenuation. Enteroviral vaccines (polio) do not appear to be good interferon inducers, which correlates with the poor interferon response elicited by this group of viruses in experimental infections in mice. Finally, vaccines containing bacterial antigens do not seem to induce interferon in man, even though various bacteria, and their products, are capable of interferon induction in experimental animals when given in doses disproportionately larger than those which could be safely given in man (13).

Summary. The purpose of this study was to extend the spectrum of vaccines which are known to induce interferon in man. The vaccines selected for this study were the commercially available live attenuated poliomyelitis vaccine type 2 and a new live attenuated influenza A/England/42/72 (H3N2) vaccine. It was hoped that if these vaccines had proven themselves to be active in interferon induction, they could be employed as nonspecific inducers of interferon (along with the other vaccines known to be good interferon inducers). Five subjects, two of whom had low or undetectable polio type 2 neutralizing antibody levels were given the standard type 2 vaccine by mouth. Even though the two individuals with low initial titer levels experienced a fourfold or greater neutralizing antibody rise and one of them shed the virus in his stool, neither they nor the remaining three volunteers developed detectable levels of interferon in their sera obtained at very closely spaced intervals from day 0 to day 25 following immunization. Fifteen subjects were given approximately $10^{7.5}$ $TCID_{50}$ of influenza A/England/42/72 (H3N2) by nasal drops. Specimens consisting of sera and nasal washings were obtained at closely timed intervals for 23 days, starting with day 3 following immunization. Three of the 15 subjects (20%) developed detectable serum interferon levels. All three of these subjects had a fourfold or greater HI antibody response. Interferon could be detected in 33.3% of the subjects in the study who had fourfold or greater HI antibody rises. No interferon was detected in nasal washings, however, It is concluded that poliomyelitis is not an interferon inducer in man. Live attenuated influenza vaccine does produce an interferon response in subjects with low initial serum antibody titers. This response is at best moderate. This latter finding also suggests that the attenuation of the strain of influenza vaccine is not due to interferon inducing potential.

References.

1. Wheelock, E.F., Proc. Soc. Exp. Biol. Med. 117, 650 (1964).

2. Wheelock, E.F., and Sibley, W.A., New Engl. J. Med. 273, 194 (1965).

3. Petralli, J.K., Merigan, T.C., and Wilbur, J.R., New Engl. J. Med. 273, 198 (1965).

4. Desmyter, J., Rawls, W.E., Melnick, J.L., Yow, M.D., and Barrett, F.F., J. Immun., 99, 771 (1967).

5. Rosenbaum, M.J., Gray, J.A., Hartmann, R., Pierce, W.E., and Peckinpaugh, R.O., in "Proceedings 6th International Congress of Chemotherapy," p. 86, University of Tokyo Press.

6. Miller, L.F., Rytel, M., Pierce, W.E., and Rosenbaum, M.J., J. Amer. Med. Ass., 185, 92 (1963).

7. Smorodintsev, A.A., Gvozdilova, D.A., Romanov, Y.A., Makarjev, G.S., Rudenko, V.I., Aksenov, O.A., and Zlydnikov, D.M., Ann. N. Y. Acad. Sci. 99, 811 (1970).

8. Voroshilova, M.D., in "Pan American Health Organization, Scientific Publication No. 226," p. 133 (1970).

9. Grossberg, S.E., New Engl. J. Med. 287, 79 (1972).

10. Rytel, M.W., and Kilbourne, E.D., Proc. Soc. Exp. Biol. Med. 137, 443 (1971).

11. Jao, R.L., Wheelock, E.F., and Jackson, G.G., J. Infec. Dis. 121, 419 (1970).

12. Murphy, B.R., Baron, S., Chalhub, E.G., Uhlendorf, C.P., and Chanock, R.M., J. Infec. Dis. 128, 488 (1973).

13. Rytel, M.W., Ferstenfeld, J.E., Rose, H.D., Balay, J., Pierce, W.E., and Lynch, K.L., Amer. J. Epidemiol., 99, 347 (1974).

Table 1. Induction of Interferon in Man by Live Poliovirus Type 2 Vaccine*

No.	Subject	Previous Polio Vaccine History	Neutralizing Antibody Response† Day	Titer	Interferon Response‡
1	R.M.	Live, trivalent 1 yr prior	0	48	None
			25	32	
2	D.D.	Live, trivalent 10 yrs prior	0	12	None
			25	>256	
3	M.N.	Live, trivalent 7 yrs prior	0	32	None
			25	26	
4	D.D.**	Never received oral	0	<4	None
			25	256	
5	S.R.	Unknown	0	54	None
			25	44	

*Given by mouth in dose of approximately $10^{4.7}$ $TCID_{50}$ on day 0 (polio vaccine monovalent, type 2, Sabin strain, Wyeth, Lot. No.17702).

†Against 100 $TCID_{50}$ of homologous strain, in WI-38 cells.

‡Tested on days 0, 2, 5, 10, 15, 20 and 25.

**Virus recovered from rectal swab on day 5.

Table 2. Induction of Interferon in Man by Live Influenza A/England/42/72 Vaccine*

No.	Subject	HI Antibody Response†		Interferon Response‡ (units/2 ml)	
		Day	Titer	Serum	Nasal Wash
1	B.T.**	0	8	None	None
		30	32		
2	D.W.	0	16	None	None
		30	16		
3	D.B.	0	<8	2 u.	None
		30	16	day 3	
4	C.S.	0	256	None	None
		30	128		
5	L.A.**	0	<8	None	None
		30	16		
6	L.L.	0	8	None	None
		30	128		
7	H.M.	0	8	None	None
		30	16		
8	P.J.	0	8	None	None
		30	8		
9	P.C.	0	<8	4 u.	None
		30	32	day 3	
10	H.P.	0	32	None	None
		30	32		
11	T.M.	0	<8	2 u.	None
		30	16	day 3	
12	D.D.	0	64	None	None
		30	64		
13	B.D.	0	8	None	None
		30	32		
14	K.G.	0	8	None	None
		30	32		
15	P.G.	0	<8	None	None
		30	16		

*Given intranasally by drops in dose of approximately $10^{7.5}$ $TCID_{50}$ on day 0 and 14 (Influenza A/England/42/72, Alice Strain, Smith Kline and French).

†Against 4 hemagglutinating units of homologous strain.

‡Tested on days 3, 7, 16 and 23. No response unless otherwise stated.

**Virus recovered in nasal wash on day 3 and 16 respectively.

Table 3. Interferon Inducing Potential of Various Vaccines in Man

Vaccine	Interferon Induction		Reference
	Systemic	Local	
Vaccinia	ND	+ (dermal crusts)	Wheelock, Proc. Soc. Exp. Biol. Med. 117:650, 1964.
Yellow fever (17-D)	+ (serum)	N.D.	Wheelock & Sibley, New Eng. J. Med. 273:194, 1965.
Measles	+ (serum)	N.D.	Petralli, et al., New Eng. J. Med. 273:198, 1965.
Measles	+ (serum) (urine)	N.D.	Desmyter, et al., J. Immunol. 99:771, 1967.
Live enteroviral vaccines	+ (serum)	N.D.	Voroshilova, PAHO Scientific Publ. #226, p. 133, 1970.
Polio	+ (serum)	N.D.	Smorodinstsev, et al., Ann N.Y. Acad. Sci. 173:811, 1970.
Mumps	+ (serum)	+ (nasal wash)	Ibid.
Influenza A_0, A_1 and B	+ (serum)	+ (nasal wash)	Ibid.
Influenza A_2	+ (serum)	+ (nasal wash)	Jao, et al., J. Infect. Dis. 121:419, 1970.
Influenza A_2	+ (serum)	+ (nasal wash)	Murphy, et al., J. Infect. Dis. 128:988, 1973.
Mixed bacterial vaccine	0	N.D.	Rytel, et al., Amer. J. Epidem. 99:347, 1974.

STUDIES OF INTERFERON ANTAGONIST(S)

An additional aspect of this study included evaluation of the possible induction of interferon antagonists by viral agents. The rationale for this was to assess whether some of the vaccines given to naval recruits could lead to release of interferon antagonists which might impair the interferon activity induced by other vaccines or in response to the naturally acquired respiratory viral infections.

Certain viruses, such as Newcastle disease virus (NDV) are excellent interferon inducers in mice; others like coxsackie B_3 produce a more meager response in the same animal species (6). It is noteworthy that NDV appears to be avirulent in mice (5), whereas coxsackieviruses B produce a severe infection associated with cytolysis (6). A number of viral infections are known to be accompanied by release of factors which inhibit interferon activity. These have been referred to as "stimulons" (1), "enhancers" (4) and "anti-interferons" (3). The last one of these appears to be of particular interest because it was isolated from Hela cells infected with the Mahoney strain of poliovirus, a poor inducer of interferon, whereas a mutant ML/15 derived from the same polio strain was a good interferon inducer and produced no "anti-interferon". Vilcek suggested that this dichotomy of effects could be due to the fact that the Mahoney strain produced enough anti-interferon to mask the presence of interferon, if any had been induced (9).

The purpose of this study was to determine whether the generally low interferon titers found in sera and organ extracts of coxsackie B_3 infected mice could be due to release during cytolysis of an interferon antagonist. Adult, random-bred Swiss mice were used. They were infected with 10^4 to 10^6 $TCID_{50}$ of coxsackie B_3 (Nancy strain) given intraperitoneally. Mice were sacrificed on day 2 and 5. Organ extracts were obtained either by the method of Fournier, et al. (2) or as supernates of homogenates of 50% weight by volume suspensions of organs in E-BME MM (Microbiological Associates, Bethesda, Maryland). Suspensions were clarified by centrifugation at 10,000 RPM's on a Sorvall RC-2 centrifuge with SM-889 head. Virus was inactivated by ultraviolet irradiation in samples studied for antagonist employing a GE germicidal lamp (G8 T5) at a distance of 7 inches for 15 minutes. Samples assayed for antagonist were added to L-929 monolayers in petri plates (Falcon Plastics) 4 hours after pretreatment with NDV-induced mouse (L-929) interferon, at which time antiviral effect of interferon had been fully established. Following an additional 18 hour incubation, monolayers were washed with phosphate buffered saline (PBS) and challenged with 100 PFU or vesicular stomatitis virus (VSV-Indiana strain). Monolayers were overlaid with 5 ml of enriched agar, reincubated for 48 hours, stained directly with 0.02% neutral red and the plaques counted. The method for assaying for antagonist activity was similar to that employed by Sheaff and Stewart in their studies of an interferon antagonist obtained in a hamster cell line (BHK-21) infected with sindbis virus (7).

It was found that an antagonist was detectable in spleen extract (obtained by either method) and sera of normal mice. Spleen extracts from infected mice also contained an antagonist provided they were free of interferon activity. No antagonist was present, however, in sera from infected mice which contained measurable amounts of interferon. Results from representative experiments are depicted in Table 1. It will be seen that there was an inverse relationship between the interferon activity ("percent reduction in VSV plaque number") and the effect of the antagonist.

We conclude that because normal mouse tissues and sera contain an interferon antagonist, the low interferon levels observed in coxsackie B_3 infected mice cannot be accounted for simply by the induction of an antagonist during the infectious process. The antagonist found in the normal mouse sera and tissue extracts could be similar to the "tissue antagonist of interferon" described by Fournier, et al. in extracts of human chorionic or amniotic membranes (2) or to the antagonist reported in normal fetal calf serum by Vilcek and Lowy (8).

References.

1. CHANY, C., and C. BRAILOVSKY: Stimulating interaction between viruses (stimulons). Proc. Nat. Acad. Sci. (Wash.) 57, 87-94 (1967).

2. FOURNIER, F., S. ROUSSET, and C. CHANY: Investigations on a tissue antagonist of interferon (TAI). Proc. Soc. Exp. Biol. Med. 132, 943-950 (1969).

3. GHENDON, YU.Z., I.G. BALANDIN, and I.M. BABUSHKINA: On the mechanism of the ability of certain viruses to block the action of interferon. Acta Virol. 10, 268-270 (1966).

4. KATO, N., F. OTA, and A. OKADA: Counteraction between interferon and enhancer. Virology 28, 785-788 (1966).

5. RYTEL, M.W., and E.D. KILBOURNE: The influence of cortisone on experimental viral infection. VIII. Suppression by cortisone of interferon formation in mice injected with Newcastle disease virus. J. Exp. Med. 123, 767-776 (1966).

6. RYTEL, M.W., and E.D. KILBOURNE: Differing susceptibility of adolescent and adult mice to non-lethal infection with coxsackievirus B_3. Proc. Soc. Exp. Biol. Med. 137, 443-448 (1971).

7. SHEAFF, E.T., and R.B. STEWART: A substance enhancing virus growth and antagonistic to interferon action. Canad. J. Microbiol. 14, 965-969 (1968).

8. VILCEK, J., and D.R. LOWY: Interaction of interferon with chick embryo cells. Arch. ges. Virusforsch. 21, 254-264 (1967).

9. VILCEK, J.: Interferon, p. 77. New York: Springer-Verlag New York, Inc., 1969.

Table 1. Reversal of Interferon Effect by Mouse Spleen Extracts and Sera

Treatment			Percent Plaque Reduction from VSV Control Plaque Number			
Interferon*	+	Antagonist†	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Interferon	+	None	50.0	70.2	93.4	93.8
Interferon	+	Infected Spleen‡ Extract	19.2	49.6	76.6	N.D.**
Interferon	+	Normal Spleen Extract	20.5	44.0	N.D.**	81.5
Interferon	+	Infected Serum Pool	56.2	64.9	90.6	N.D.**
Interferon	+	Normal Serum Pool	9.4	47.4	N.D.**	51.0

*Mouse interferon induced in L-929 cells by NDV was added to L-929 plates 4 hours before antagonist. Plates were then incubated for 18 hours and challenged with VSV (Indiana Strain) approximately 100 PFU.

†Pooled preparations from 5 mice.

‡50% w/v suspension made in BME MM; clarified by centrifugation 10,000 RPM/20 min.; UV irradiated (as was infected serum) 7" distance/15 min.

**Not done.