Persistence and Spread of Influenza Virus (A2/Hong Kong) in Normal and Poly I.C. Treated Baboons (*Papio cynocephalus*)[1] (35128)

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Recent advances in the development of inducers for *in vitro* and *in vivo* formation of interferon have given hope that viral diseases may be prevented or modified. Park and Baron (1) demonstrated this possibility in rabbits with keratoconjunctivitis induced by herpes simplex virus. Treatment with polyinosinic:polycytidilic acid copolymer (poly I.C.) altered the course of disease when given as long as 3-days postinfection. Other studies have shown interferon inducers to be effective in small animals but work with nonhuman primates has been limited. Cochran *et al.* (2) showed a statolon-like preparation was effective in preventing or delaying paralysis in cynomolgus monkeys due to poliovirus type II. Kleinschmidt and Murphy (3) demonstrated that statolon induced circulating interferon in African green monkeys. Despite this, it has been felt that nonhuman primates are poor producers of interferon. Their close relationship to man, however, suggests results obtained from the study of these animals would more accurately reflect the usefulness of interferon inducers in combating human disease.

Our recent demonstration of the susceptibility of baboons to a Hong Kong strain of influenza virus A2 (4) prompted us to study the effect of poly I.C. on the course of this disease in experimentally infected baboons and their contacts. In this previous study, inoculated animals were found not to be excreting virus at the end of the 11-day sampling period. One of the naturally infected controls, however, was positive. This presented the possibility that virus excretion may have persisted beyond this time. Since the persistence of virus excretion is an important parameter in the spread of infection, our original observations were extended to determine more accurately how long influenza virus may be isolated from the throats of infected baboons.

TABLE I. Data on Experimental Baboons.

Animal	Sex	Wt (kg)	Treatment
Expt. 1			
A	Male	1.79	Influenza virus
B	Female	2.64	Influenza virus
C	Female	1.64	Influenza virus
D	Female	3.26	Control
E	Female	3.17	Control
F	Male	1.67	Control
G	Female	0.80	Control + poly I.C.
H	Male	0.79	Control + poly I.C.
Expt. 2			
I	Male	3.26	Influenza virus
J	Male	4.71	Influenza virus
K	Male	4.47	Control
L	Female	3.82	Control
M	Male	1.92	Influenza virus + poly I.C.
N	Male	1.10	Influenza virus + poly I.C.
O	Male	1.60	Control + poly I.C.
P	Female	0.88	Control + poly I.C.

Materials and Methods. Animals. The baboons (*Papio cynocephalus*) used in these experiments were imported from Africa or born at the Southwest Foundation for Research and Education. Animals used in this study are described in more detail in Table I. They were tranquilized with Sernylan (Parke, Davis, and Co.) prior to inoculation and in Expt. 1 lightly anesthetized with ether. Collection and handling of these animals in Africa or at this facility has been de-

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TABLE II. Development of HI and CF Antibodies in Uninoculated and Influenza Virus Inoculated Baboons and Poly I.C. Treated Controls (Expt. 1).

Day sampled	Baboon treatment															
	Influenza inoculated						Uninoculated controls						Poly I.C. controls			
	A		B		C		D		E		F		G		H	
	HI	CF	HI	CF	HI	CF	HI	CF	HI	CF	HI	CF	HI	CF	HI	CF
0	—[a]	—	—	—	—	AC[b]	—	<20	—	10	—	—	—	—	—	—
7	—	—	—	10	—	AC	—	<20	—	<20	—	AC	—	—	—	—
14	80	20	40	20	—	AC	—	<20	—	—	—	—	—	—	—	—
25	40	20	10	10	—	AC	20	20	40	20	40	20	—	—	—	—
35	80	10	10	10	80	20	10	40	20	10	—	10	—	—	—	—
43	80	10	40	10	80	AC	20	40	20	20	40	20	—	—	—	—
67	40	10	20	10	20	AC	10	20	20	10	40	10	—	—	—	—
92–101	40	—	20	—	20	—	10	—	40	—	20	—	—	—	—	—

[a] — = <10.

[b] AC = anticomplementary.

scribed previously (5–8). All the animals in an experiment were handled as a group and kept individually in adjoining cages.

Specimen handling. Specimens were collected and processed for virus isolation and serology at intervals indicated in Tables II–V. The methods employed have been described previously (4, 9).

Virus. A Hong Kong strain of influenza virus (A2/Phila.101/68), supplied by Dr. F. S. Lief, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, as a second egg passage from its human donor was used for the baboon inoculum. It had a hemagglutination (HA) titer of 1:320 per 0.4 ml and a titer of $10^{4.5}$ EID_{50} per 0.1 ml. Animals were inoculated by the intranasal instillation of 0.5 ml of undiluted chicken embryo allantoic fluid containing virus.

Poly I.C. treatment. A different lot of poly I.C. was used for each of the two experiments. Experiment 1 was carried out with a preparation supplied by Dr. Samuel Baron, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. It contained 1 mg of poly I.C./ml 0.85% NaCl, pH 7.8. Poly I.C. purchased from P-L Biochemicals, Milwaukee, Wisconsin (Catalogue #4712, Lot 2273) was used in Expt. 2. Baboons receiving poly I.C. were inoculated with 6 mg/kg of body weight by the intravenous route and 1 mg/kg intranasally at the time of infection. The intranasal dose was repeated on days 2 and 4. At no time was a toxic reaction noted in any of the treated animals.

Interferon assay. Baboon serum was tested for interferon content on baboon kidney cell cultures in Micro Test II tissue culture plates (Falcon Plastics, Oxnard, California). Inhibition of cytopathic effects of 100 $TCID_{50}$ vesicular stomatic virus, after overnight incubation with serum dilutions on the cells, was used to score the assay. A 50% difference between control virus and virus on serum treated cells was considered a positive test.

Results. Expt. 1. Three baboons were inoculated with influenza virus and quartered with 5 control animals, 2 of which were treated with poly I.C. Table II illustrates the development of hemagglutination inhibition (HI) and complement fixing (CF) antibodies. Two of the 3 inoculated animals had detectable HI antibody by the end of the second week. The third became positive between the fourth and fifth weeks. The 3 untreated control animals all developed HI antibody by the 25th day, but neither of the animals treated with poly I.C. became positive. Where detectable, CF antibody formation paralleled or preceded HI antibody. It also appears to decay before HI antibody.

Influenza virus was isolated from the 3

TABLE III. Influenza and Simian Adenovirus Isolation from Expt. 1 Baboons.

	Baboon treatment															
	Influenza inoculated						Uninoculated controls						Poly I.C. controls			
	A		B		C		D		E		F		G		H	
Day sampled	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno
0						S										
2	+[a]				+							S		S		
4	+							S				S		T		
7			+			S		S								
10		S[b]			+	S			+			S				
14			+	S		S	+			S		S				
16		S								S		TS			+	
21												S				
23				S								S				
25					+							S				
28														S		S
35																
43				S		S										S
53		S														
60								S		S				TS		S
67		S		TS		TS		TS		TS		TS		TS		TS

[a] Influenza A2/Hong Kong isolates, blank spaces negative.

[b] S = Simian adenovirus stool isolate; T = Simian adenovirus throat isolate.

TABLE IV. Development of HI and CF Antibody in Uninoculated and Influenza Virus Inoculated Baboons Treated with Poly I.C. (Expt. 2).

	Baboon treatment															
	Virus inoculated				Uninoculated controls				Virus inoc. + poly I.C.				Uninoc. cont. + poly I.C.			
	I		J		K		L		M		N		O		P	
Day sampled	HI	CF	HI	CF	HI	CF	HI	CF	HI	CF	HI	CF	HI	CF	HI	CF
0	—[a]	<20	—	<20	—	—	—	—	—	—	—	—	—	—	80	—
7	10	<20	—	—	—	—	—	—	10	—	—	—	—	—	40	—
14	80	<20	20	10	—	—	—	—	—	—	—	—	—	—	—	—
21	40	<20	20	10	80	20	40	10	—	—	20	—	—	—	—	—
28	320	<20	80	—	160	20	80	10	—	—	80	—	—	—	—	—
37	320	<20	80	10	160	10	80	10	10	—	160	—	—	—	—	—[b]
42	160	<20	160	10	80	10	80	10	20	—	320	—	—	—		
49	320	<20	320	10	320	10	320	10	10	—	320	—	—	—		
57–106	80	—	20	—	20	—	20	—	—	—	ND[c]		—	—		

[a] — = <10.
[b] Died on day 40.
[c] ND = not done.

inoculated baboons, 2 of the 3 controls and 1 of the 2 poly I.C. treated animals (Table III). The last day of isolation from the inoculated baboons varied from the 4th to the 25th day. It may be significant that no HI antibody was detected until after virus excretion stopped in the animal excreting virus over the longest period of time (C). Animal B, however, did have antibody on day 14 when virus was isolated. Controls were excreting virus on days 10 (E) and 14 (D) and virus was isolated from the poly I.C. control (H) at approximately the same time (16th day).

Expt. 2. In this experiment 4 baboons were inoculated with virus and quartered with 4 control animals. Two baboons in each group were treated with poly I.C. The 2 untreated, inoculated baboons developed HI antibody during the first and second weeks (Table IV). The untreated controls followed with antibody during the third week. In both groups of animals the HI titer decreased during the 57th to 106th days.

Of the 2 inoculated baboons treated with poly I.C. animal N responded with antibody formation during the second week, like an uninoculated control. Animal M produced only a low level of antibody during the fifth to seventh weeks after inoculation.

One poly I.C. treated control animal (O) produced no HI antibody. Sera from the other treated control (P) appeared to contain a nonspecific inhibitor at several bleedings, including days 0, 7, and 28. Other sera collected on days 2, 14, 21, and 37 had HI titers of <1:10. This animal died on day 40 of an apparent *Staphylococcus aureus* bacteremia.

One of the 2 influenza virus inoculated and the 2 uninoculated control animals produced detectable CF antibody at the same rate as HI antibody. Sera from animal I reacted nonspecifically at a 1:10 dilution. All of the poly I.C. treated animals were negative at a 1:10 dilution despite the presence of HI antibody at a titer of 1:320 in animal N.

Virus was isolated from every animal except animal 0 on day 2 (Table V). In the influenza virus inoculated baboons, virus was isolated on days 2 and 4. In the inoculated poly I.C. treated animals virus was isolated on day 7 in one instance. Virus persisted in the controls for 11 days in the absence of poly I.C. treatment and 7 days with treatment.

Sera collected on days 0, 2, 4, and 7 from

TABLE V. Influenza and Simian Adenovirus Isolation from Expt. 2 Baboons.

Day sampled	Baboon treatment															
	Virus inoculated				Uninoculated control				Virus inoculated + poly I.C.				Uninoculated control + poly I.C.			
	I		J		K		L		M		N		O		P	
	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno	Flu	Adeno
0										S		T				S
2	+[a]	S[b]	+		+		+	TS	+	S	+	TS		TS	+	TS
4	+	S	+	S	+		+	S		S				S		S
7		S						TS	+	S			+	TS	+	TS
11		S			+	S				S		S		S		TS
14						S				S		TS		TS		TS
18												TS		S		TS
21												TS				T
28																
32																
35																Dead

[a] Influenza A2/Hong Kong isolates.

[b] S = simian adenovirus stool isolate; T = simian adenovirus throat isolate.

baboons A and D–P were tested for the presence of interferon. All had a titer of $<1:10$.

Isolation of viruses indigenous to the baboon. In both experiments a number of simian adenovirus isolates were made (Tables III and V). Most of these isolates were one of the following: SV25, SV33, SA7, or V340. As many as 3 serotypes were isolated from a single baboon during the observation period. There appears to be no relationship between the excretion of these viruses and the course of the influenza virus infection, nor does poly I.C. appear to influence the excretion of these adenoviruses. No attempt was made to determine the amount of virus being excreted, however, and incomplete inhibition by interferon would go undetected.

Discussion. These findings confirm our earlier observations. The baboon is readily infected by influenza virus (A2/Phila.101/6) following intranasal instillation of the virus or by natural transmission via the airborne route. HI and CF antibodies generally develop within 2 weeks of infection and begin to decline after the seventh week. Virus can be isolated from nasal and throat washings. Virus was isolated from one baboon as long as 25 days postinoculation, indicating influenza virus transmission from animal to animal may be possible over a much longer period of time than generally thought possible.

Treatment of baboons with poly I.C. at the time of inoculation with influenza virus appears to delay or suppress infection in terms of the formation of HI and CF antibody. When uninoculated sentinel animals are treated with poly I.C. antibody formation is not observed, suggesting the natural transmission of disease is altered. Isolation of virus from poly I.C. treated control baboons suggested further that infection is not completely suppressed and may be continuing at a superficial or limited level in the nasal mucosa. In any event, the amount of virus present is too small or incapable of stimulating antibody formation. Thus, poly I.C. may be successful in suppressing or mitigating influenza virus infections in the treated indi-

vidual while allowing transmission to untreated contacts. The decreased effect of poly I.C. in the experimentally infected baboons, as compared to treated control animals, is likely to be a dose response. The inoculated animals probably receive more infectious virus particles than the naturally infected controls. Treatment with poly I.C. prior to inoculation of virus may cause complete suppression of infection by allowing time for interferon to be synthesized.

Lack of CF antibody in the presence of HI antibody in animals treated with poly I.C. (baboons M and N) is likely to be a reflection of the comparatively low CF titers achieved by influenza virus infected baboons rather than specific inhibition by poly I.C. Therefore, the HI test seems to be a more sensitive indicator of infection.

In a preliminary study, conducted in collaboration with Dr. Samuel Baron, circulating interferon was detected in the baboon up to 24 hr after the intravenous inoculation of 1 mg/kg of poly I.C. At 48 hr no interferon was detected. This is similar to the results of Kleinschmidt and Murphy (3), using statolon in African green monkeys. Intratracheal instillation of the same amount of poly I.C. in the baboon failed to induce any circulating interferon. This suggests the protective effect of poly I.C. persists long after the dsappearance of interferon and may be related to the binding of interferon to tissue sites. If this is true, it is not apparent which route of inoculation, intravenous or intranasal, contributed more to the observed effect of poly I.C. in these experiments. Failure to detect circulating interferon in any of the animals tested may be a reflection of the time serum samples were collected. The first of these was obtained at 48 hr after the initial inoculation of poly I.C. Previous results indicate that interferon levels have dropped to $<1:5$ by this time. Subsequent bleedings were at 48- to 72-hr intervals following intranasal instillation of poly I.C., although this route of poly I.C. administration is probably ineffective in stimulating circulating interferon. This is indicated by the results obtained with intratracheal inoculation of poly I.C. Although Jao *et al.* (10) were able to demonstrate circulating interferon in human volunteers infected with an Asian strain of influenza A2 virus during the period of virus excretion, none was detected in the infected baboons. Again this may be a reflection of low interferon titers, improper sampling or bound interferon.

Several studies have demonstrated that mice and humans can be protected from influenza virus infections by the inoculation of preformed interferon (11, 12) or inducers such as inactivated influenza virus (13) and nucleic acids (14). *In vivo* studies with influenza virus strains, including A2/Hong Kong, in mice have not demonstrated a remarkable effect of poly I.C. in preventing infection (15, 16). It is difficult to correlate these findings with those in the baboon, however, because effectiveness of poly I.C. in mice is defined in terms of mouse survival and not virus excretion or antibody formation, the parameters used in this study. None of the baboons studied showed unequivocal evidence of influenza disease, although a slight nasal discharge was noted in the infected animals in Expt. 2. The irregular isolation of virus leaves antibody formation as the most consistent indication of infection. The ease with which control animals became infected (8/8 in 3 experiments) suggests they would be good indicators of the infectiousness of treated and untreated baboons over a period of time if introduced to the group at staggered intervals. Reliance on antibody formation as indication of infection, however, remains the simplest approach.

Although a number of simian adenovirus isolates were made during the course of these experiments, they did not seem to influence the findings in any consistent manner. Of the adenovirus serotypes isolated, V340 has been associated with pneumoenteritis in baboons (17). SV25, SV33, and SA7 are more frequently isolated from stool specimens of the baboon and have not been associated with upper respiratory disease (18). V340 was isolated most frequently after the first 2 weeks of the experiment, but this probably has no association with poly I.C. treatment. In general, poly I.C. seems to have no effect on the adenovirus infections. No antibody studies have been done, however, to deter-

mine the effect on this parameter nor was the quantity of virus excretion monitored.

The number of animals used in these experiments is rather small but the results are consistent enough to be encouraging. It seems to us that systems such as these should be used for the study of human virus diseases and their alteration by prophylactic or therapeutic drug if more meaningful data are to be derived.

Summary. Baboons inoculated intranasally with influenza virus A2/Phila.101/68, a Hong Kong strain, excreted virus for as long as 25 days. Infection was associated with seroconversion and transmission to uninoculated control animals. The control animals also excreted virus and showed seroconversion. Treatment of inoculated baboons with poly I.C. delays or suppresses antibody formation and prevents it in uninoculated contact animals. In neither case is virus excretion consistently prevented.

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