INFECTION AND IMMUNITY, July, 1975, p. 62-68

Vol. 12, No. 1
Printed in U.S.A.

Failure of Attenuated Temperature-Sensitive Influenza A (H3N2) Virus to Induce Heterologous Interference in Humans to Parainfluenza Type 1 Virus

BRIAN R. MURPHY,* DOUGLAS D. RICHMAN, ELIAS G. CHALHUB, CAROL P. UHLENDORF, SAMUEL BARON, AND ROBERT M. CHANOCK

Laboratory of Infectious Diseases, and Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014, and Barnes Hospital, St. Louis Missouri 63110*

Received for publication 14 March 1975

The present investigation was undertaken to determine if a candidate live vaccine virus, influenza A/Hong Kong/68-*ts*-1[E] (H3N2), induced heterologous interference against an interferon-sensitive, wild-type, parainfluenza type 1 challenge virus. The parainfluenza virus was administered 7 days after Hong Kong/68-*ts*-1[E] virus infection. The clinical response, daily quantitative virus shedding, interferon production, and serum and nasal wash antibody responses were determined in an experimental group (influenza A virus followed by parainfluenza virus) and 10 volunteers in a control group (parainfluenza virus only). The volunteers were selected on the basis of susceptibility to the two viruses, i.e., serum hemagglutination-inhibition antibody titer of $\leq$1:8 for influenza virus and low nasal wash antibody titer ($\leq$1:8) for parainfluenza virus. Despite a 100% infection rate in the Hong Kong/68-*ts*-1[E] vaccinees, no heterologous interference was induced against the parainfluenza type 1 virus challenge.

A potential advantage of a live virus vaccine might lie in its ability to stimulate early nonspecific resistance to related or unrelated viruses in addition to immunologically specific resistance. Practical usefulness of such nonspecific resistance would be in the prevention of the selected infections such as nosocomial infections with homologous as well as heterologous wild-type viruses (C. B. Hall and R. G. Douglas, Pediatrics, in press). Nonspecific resistance in humans between vaccine and wild-type virus has been observed in many situations (25). Interference between viruses that replicate in the respiratory tract has been reported (3, 5, 6, 8, 9, 22, 23) but has not been observed in all instances (13, 14, 22).

We sought to determine if a live, temperature-sensitive vaccine candidate influenza A virus, influenza A/Hong Kong/68-*ts*-[E] (H3N2), which stimulated low levels of interferon in nasopharyngeal secretions of man (15), could provide nonspecific resistance to a heterologous, interferon-sensitive virus, parainfluenza type 1 (11). The parainfluenza type 1 virus was given 7 days after administration of the influenza virus to test whether heterologous resistance was present early in the course of immunization when one would be most likely to detect it (8).

METHODS AND MATERIALS

Viruses. The parainfluenza type 1 virus was isolated in primary African green monkey kidney (AGMK) tissue culture from a child with an upper-respiratory-tract illness. Primary monolayer cultures of AGMK were prepared in 32-ounce (ca. 0.95 liters) bottles using as growth media 50 ml of H-Lac (0.5% lactalbumin hydrolysate in Hanks balanced salt solution [HBSS]) with 2% fetal bovine serum, free of antibiotics. The bottles were incubated at 37 C, refed 3 days later with 50 ml of media, and incubated for an additional 4 days before virus inoculation with 1 ml of AGMK passage 2 seed virus containing 10 mean tissue culture infective doses ($TCID_{50}$)/ml of parainfluenza type 1 virus. After a 2-day incubation at 35 C, the bottles were washed twice with 100 ml of HBSS and refed with 100 ml of medium 199 (Flow Laboratories, Rockville, Md.) containing 10% of a 1$\times$ concentration of SPGA (sucrose, 0.218 M; KH_2PO_4, 0.0038 M; K_2HPO_4, 0.0072 M; potassium glutamate 0.0049 M; and human serum albumin, 1% [2]) with 50 μg of neomycin and 10 μg of chlortetracycline-hydrochloride per ml. The cultures were reincubated at 35 C for an additional 5 days at which time no cytopathology was apparent in infected or control cultures, but an infected culture showed confluent hemadsorption when tested with guinea pig erythrocytes. The cells were harvested, subjected to 1 rapid-freeze-thaw cycle in an attempt to release cell-associated virus, pooled, and refrozen at -80 C before clarification. To 1,600 ml of crude thawed fluid was added 2 g of celite no. 503 (Johns Manville, Denver, Colo.) suspended in 16 ml of

sterile distilled water. This suspension was then filtered through a coarse glass filter (1 by 8 inch; ca. 2.5 by 20 cm) which had been primed with a mixture of celite no. 503 (10 g) and 0.05% gelatin in 800 ml of sterile distilled water. A 10× concentration of SPGA was added 1:10 to the filtrate, and the fluid was again shell frozen before final storage at −140 C. The virus suspension was found free of adventitious agents by testing procedures previously described (12, 16). The virus which titered $10^{6.5}$ $TCID_{50}$/ml in AGMK roller tube culture was diluted 30-fold in medium L-15 before being administered intranasally (1 ml) as a coarse droplet spray using a no. 15 DeVilbis nebulizer.

The isolation, passage history, preparation, safety testing, administration, and clinical testing of the allantoic grown influenza A/Hong Kong/68-ts-1[E] recombinant virus was described previously (17). Volunteers received $10^{7.0}$ $TCID_{50}$ intranasally by DeVilbiss no. 15 coarse droplet atomizer. The results are presented here again for the purpose of orientation.

The Sindbis virus (used in the interferon assay) was passaged multiple times in primary chicken embryo fibroblast monolayer cultures and titered $10^{8.0}$ plaque-forming units/ml on chicken embryo fibroblast monolayer cultures.

Clinical studies. The present study was carried out at the Lorton Reformatory in Lorton, Va. Informed consent was obtained from each volunteer prior to his participation in the study. To insure susceptibility to the parainfluenza 1 virus challenge, volunteers were selected on the basis of a low level of nasal wash antibody ($\leq$1:8) to parainfluenza type 1 virus regardless of serum antibody level (21). These volunteers were further subdivided into two groups of 10 men each: (i) one group with low levels of serum hemagglutination-inhibition (HI) antibody ($\leq$1:8) to influenza A/HK/68 virus (H3N2) received the *ts*-1[E] virus vaccine followed by parainfluenza type 1 virus challenge 7 days later, and (ii) a control group with higher levels of serum HI antibody to influenza A/HK/68 virus (>1:8) who received only parainfluenza type 1 virus. Parainfluenza type 1 virus was administered 7 rather than 2 or 3 days (time of peak interferon titer) after the Hong Kong/68-*ts*-1[E] virus for the following three reasons. (i) The reactogenicity and the genetic stability of the influenza *ts* virus would be obscured if the parainfluenza virus was administered sufficiently early that both viruses would be replicating simultaneously; (ii) if the period of resistance to a heterologous virus were not evident 7 days after administration of Hong Kong/68-*ts*-1 [E] virus, then the chance for this type of resistance to have practical applications would be small; and (iii) previous work had demonstrated heterologous resistance at 2 and 5 weeks after a specific respiratory virus infection (8). Although the groups were not matched identically for serum HI antibody to influenza A virus, this was considered an advantage because it would tend to prevent unwanted spread of the *ts*-1[E] virus from the vaccinees to the controls. After 3 days in isolation in a dormitory, the experimental group received $10^{7.0}$ $TCID_{50}$ of the *ts*-1[E] vaccine virus intranasally. Seven days later the control group was admitted to the same dormitory, and on that day both groups received $10^{5.0}$ $TCID_{50}$ of the parainfluenza type 1 virus intranasally. The control group was admitted on day 7 to minimize the chance of spread of virus from the *ts*-1[E] virus vaccinees to the control group. In fact, no shedding of the *ts*-1[E] virus was detected after day 5 postinoculation.

For the first 7 days, nasopharyngeal wash specimens were collected daily in veal infusion broth containing 0.5% gelatin. Each specimen was inoculated into four primary rhesus monkey kidney culture tubes in an attempt to recover influenza virus. Similary, after parainfluenza type 1 virus administration, each nasopharyngeal wash specimen was inoculated into four primary AGMK culture tubes to recover parainfluenza virus. For interferon determination, daily nasopharyngeal wash specimens were collected with 5 ml of serum-free Eagle no. 2 medium into each nostril followed by gargle with 10 ml of medium. This procedure was repeated 30 min later, and the collections were pooled. Antibiotics were added to the pooled nasopharyngeal specimen to achieve a final concentration of 250 U of penicillin, 250 μg of streptomycin, and 50 U of amphotericin per ml. Blood (40 ml) was collected for serological studies before administration of parainfluenza virus and at weekly intervals thereafter for 4 weeks. Weekly saline nasal washings also were collected and concentrated with Aquacide (Calbiochem). Concentrated nasal washings were adjusted to 20 mg of immunoglobulin A/100 ml before being tested for antibody titer.

Measurement of infectivity, neutralizing antibody, and HI response. Infectivity was determined by the hemadsorption technique using rhesus monkey kidney tissue culture tubes for influenza virus and AGMK for parainfluenza virus. Neutralizing antibody was measured in rhesus monkey kidney tube cultures for both viruses by the techniques previously described (22). Nasal wash antibody was measured by using the neutralization test.

Interferon determination. Interferon was assayed by the method of yield inhibition of Sindbis virus hemagglutinin in human foreskin fibroblast culture (Biofluids HFS-1 strain at 10th to 15th passage) as previously described (18). Nasopharyngeal (NP) wash specimens that were collected in Eagle no. 2 media were applied to the monolayer undiluted or at 1:5 and 1:50 dilutions for assay. NP wash specimens that were collected in veal infusion broth containing 0.5% gelatin were assayed at a starting dilution of 1:5. The interfering activity detected in NP wash specimens had been characterized previously (15). The specimens collected in this study possessed the following characteristics of interferon: (i) extensive washing of cultures which have been preincubated with NP washings failed to reverse interfering activity against Sindbis virus challenge and (ii) stability at pH 2 for 48 h.

Determination of interferon sensitivity of parainfluenza type 1 virus. Primary human embryonic kidney tube cultures (Flow Laboratories) were treated with varying concentrations of human interferon diluted in medium consisting of 1 part medium 199 and 1 part Eagle no. 2 (National Institutes of Health media production unit) with 250 U of penicillin, 250 μg of streptomycin, and 50 U of amphotericin per ml. The human interferon was prepared for the Antiviral

Substances Program, National Institute of Allergy and Infectious Diseases, by K. Cantell from human leucocytes stimulated with Sendai virus (4). After incubation for 24 h at 37 C, the tubes were washed with HBSS and inoculated with 0.2 ml of virus suspension containing 10^5 $TCID_{50}$/ml of parainfluenza type 1 virus or $10^{7.4}$ plaque-forming units of Sindbis virus. After 2 h of incubation at room temperature the tubes were washed four times with medium, leaving 1.5 ml in each tube. The human embryonic kidney tube cultures challenged with Sindbis virus were harvested 24 h after virus inoculation and those with parainfluenza virus 48 h after inoculation. The amount of virus present in the tube culture challenged with Sindbis virus was determined by measuring the amount of hemagglutinin produced (18). The amount of parainfluenza virus was determined by the $TCID_{50}$/ml titer in rhesus monkey kidney tube cultures. A reduction in virus yield resulting from the antiviral action of interferon of 0.5 $\log_{10}$ was considered significant.

RESULTS

Sensitivity of parainfluenza type 1 virus to interferon. Heterologous interference is believed to be mediated in part by the antiviral action of interferon. The interferon sensitivity of the challenge virus, parainfluenza 1 virus, thus needed to be confirmed (11). This was determined by measuring, in the same experiment, the ability of varying amounts of human interferon to reduce the yield of parainfluenza 1 virus and Sindbis virus, a virus known to be sensitive to human interferon (18). As shown in Table 1, parainfluenza 1 virus was clearly as sensitive to human interferon as Sindbis virus and, therefore, represented an interferon-sensitive virus challenge.

Infection of volunteers with influenza A/Hong Kong/68-ts-1[E] virus. The results of the intranasal administration of $10^{7.0}$ $TCID_{50}$ of the egg-grown *ts*-1[E] virus have been presented as part of another study (17) and will only be summarized here (Fig. 1). Importantly, all 10 volunteers were infected as indicated by shedding of vaccine virus and/or a fourfold or greater rise in serum antibody titer. In addition, the geometric mean serum and nasal wash antibody on day 21 was three-fourths that induced by a virulent wild-type virus. These results indicate that the volunteers underwent a moderately extensive infection with the *ts*-1[E] vaccine virus and thereby constituted an adequate group to study the question of heterologous interference by a vaccine virus. However, interferon could not be detected in the nasopharyngeal washes of these volunteers, whereas it was detected previously in 50% of another group of volunteers who received $10^{6.0}$ $TCID_{50}$ of bovine-kidney-grown virus (18). Both undiluted washes collected with Eagle no. 2 medium treated with acid to pH 2 and other washes collected in veal infusion broth diluted 1:5 and treated with anti-influenza A/HK/68 antisera (15) failed to yield detectable interferon activity in the present study, although the laboratory reference interferon yielded the expected titer. Since the level of interferon induced by *ts*-1[E] virus previously reported was low (geometric mean titer = 2.3 U/ml) and was detected in only 50% of volunteers (15), it is not surprising that it could not be detected in this group even though the lower limit of detectability of nasal wash interferon was 1 U of interferon per ml of nasal wash.

TABLE 1. *Sensitivity of parainfluenza type 1 virus and Sindbis virus to human interferon in primary human embryonic kidney tube cultures*

Interferon concn (U/ml)	Reduction in yield of parainfluenza virus[a] ($\log_{10}$/ml)	Reduction in yield of Sindbis virus[b] ($\log_{10}$/ml)
None	0	0
3	0.5	0.6
10	1.2	>0.9
30	3.0	>0.9
100	>3.8	>0.9

[a] Infectious yield of parainfluenza virus determined by hemadsorption technique in tube cultures of rhesus monkey kidney tissue.

[b] Yield of Sindbis virus determined by hemagglutinin production.

Response of the ts-1[E] vaccinees and a control group to parainfluenza 1 virus. The clinical response of the *ts*-1[E] vaccinees and of the controls to the intranasal administration of $10^{5.0}$ $TCID_{50}$ of parainfluenza type 1 virus given intranasally is presented in Table 2. It appeared that the two groups responded clinically in a similar manner to the parainfluenza type 1 virus. There were no statistically significant differences between *ts*-1[E] vaccinees and controls in number of men ill, total number of days of illness, day of onset of illness, average duration of illness, number of men with fever, and total number of days of fever. These results indicate that a previous infection with influenza A/Hong Kong/68-*ts*-1[E] virus did not modify the clinical response to parainfluenza virus infection given 7 days after administration of the *ts*-1[E] virus.

The patterns of parainfluenza virus shedding and interferon response of *ts*-1[E] vaccinees and controls are presented in Fig. 1. All volunteers shed virus, with the peak day of shedding in both groups occurring 3 days after virus administration. There was no statistically significant difference in the amount of virus shed between the two groups on any single day or over the

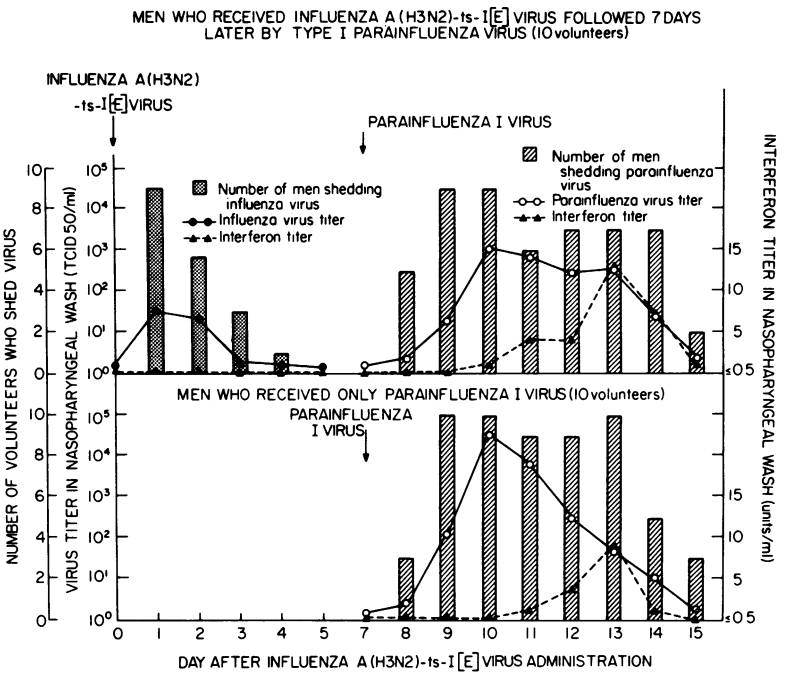

FIG. 1. *Virus shedding and interferon response after Hong Kong/68-ts-1[E] and parainfluenza type 1 virus infections.*

TABLE 2. *Comparison of illness caused by parainfluenza virus in ts-1[E]-vaccinated[a] and control[b] volunteers*

Group	No. of men	No. ill	Total no. of days of illness	Avg day of onset of illness[c]	Avg duration of illness (days)[c]	No. with fever	Total no. of days of fever
ts-1[E] vaccinees	10	6	33	2.8	5.5	3	3
Controls	10	8	42	3.4	5.2	2	6

[a] Parainfluenza type 1 virus administered 7 days after infection with ts-1[E] virus.
[b] Received only parainfluenza type 1 virus.
[c] Calculated using data only from men who developed illness.

total 8-day period. The pattern and amount of virus shedding thus was not influenced by prior infection with the *ts*-1[E] vaccine virus. The pattern and amount of the nasal wash interferon response was similar in both vaccine and control groups. It is noteworthy that the peak interferon titer occurred several days after the peak virus titer.

The serum and nasal wash neutralizing-antibody response to parainfluenza 1 virus in controls and vaccinees is presented in Table 3. Baseline nasal wash neutralizing antibody, which correlates with susceptibility to parainfluenza type 1 virus (21), was similar in both groups. Ninety percent of the volunteers had an immunological response, and no statistically significant difference between vaccine and control group was noted regarding either the number of men responding or the height of response in serum or nasal wash.

DISCUSSION

The present study was undertaken to determine if the influenza A/Hong Kong/68-*ts*-1[E] virus induced local nonspecific resistance to an interferon-sensitive, but immunologically unrelated, virus, parainfluenza type 1. A prompt and nonspecific protective effect of live vaccine virus administration against homologous wild-type virus would be particularly useful during a pandemic caused by a new antigenic subtype of influenza A virus. However, an infection with

TABLE 3. *Comparison of serum and nasal wash neutralizing parainfluenza type 1 virus antibody in ts-1[E] virus-vaccinated[a] and control[b] volunteers*

Group	No. of men	No. with fourfold or greater rise in antibody in:			Reciprocal of geometric mean neutralizing antibody titer on indicated day									
		Nasal wash	Serum	Either	Nasal wash					Serum				
					0	7	14	21	28	0	7	14	21	28
ts-1[E] vaccinees	10	8	10	10	1.5	ND[c]	ND	11	18	23	ND	ND	170	322
Control	10	8	7	8	1.8	3.4	6.5	34	61	59	48	119	188	276

[a] Parainfluenza type 1 virus administered 7 days after infection with ts-1[E] virus.
[b] Received only parainfluenza type 1 virus.
[c] ND, Not done.

the influenza A/Hong Kong/68-*ts*-1[E] virus, which previously had been shown to induce strong homologous resistance (16), failed to provide any detectable heterologous resistance when given 7 days prior to challenge with parainfluenza type 1 virus.

If interferon is one of the major mediators of local nonspecific resistance, it is not surprising that the Hong Kong/68-*ts*-1[E] virus which stimulated low (15) to undetectable levels (in the present study) of interferon provided no protection against a heterologous virus challenge. In the previous study, the peak interferon response to the Hong Kong/68-*ts*-1[E] vaccine virus was detected 2 days after virus administration (15). It is also possible that by day 7, the time of parainfluenza type 1 virus challenge, the antiviral affect of any undetected interferon had waned considerably (7), and therefore provided little protective effect. It is known that the protective effect of interferon in animals can be overcome by increasing the dose of virus challenge (1). The failure to demonstrate any protective effect under the conditions employed in the present study also might be attributable to the administration of too large a dose of parainfluenza type 1 virus in the challenge inoculum. The 50% disease-producing dose for parainfluenza 1 virus in adult volunteers has been reported to be as low as 10^2 $TCID_{50}$ with a 6-day incubation period (19), and the present challenge dose was $10^{5.0}$ $TCID_{50}$, a 100% infective dose which produced illness in 80% of control volunteers with an incubation period of 3 days. The Hong Kong/68-*ts*-1[E] virus, therefore, might have exerted a protective effect if the time from Hong Kong/68-*ts*-1[E] virus administration to parainfluenza type 1 virus challenge was less than 7 days, or if the quantity of parainfluenza virus in the challenge inoculum was decreased. If other mechanisms of nonspecific interference exist, as has been suggested (6), it is clear they were not operative under the conditions of the present experiment.

Another explanation for the failure to detect heterologous interference in the present experiment is that such interference is not a universal phenomenon applicable to all virus-virus interactions (10, 20). In man, six controlled studies have been performed in volunteers to examine the question of the induction of heterologous interference by respiratory viruses, and it was apparent that such interference was not induced in all instances. Fleet et al. demonstrated that heterologous resistance to two wild-type rhinovirus subtypes, 8 and 23, was present 2 and 5 weeks after primary virus inoculation but was not detectable at 16 weeks (8). Cate et al. extended these observations by demonstrating that wild-type rhinovirus type 15 induced resistance to Coxsackie A21 wild-type virus challenge given 4 weeks after the primary infection and that this resistance may not have been mediated by interferon (6). However, Matthews et al. were not able to detect the presence of heterologous interference between intranasally administered live enterovirus vaccine (echovirus type 1) and rhinovirus type 4 given 2 days after administration of the enterovirus (13). E. Chalhub, P. Wright, and R. Chanock (unpublished observations) were not able to document heterologous resistance between a low-temperature-adapted parainfluenza type 1 virus, which caused mild illness in 13 of 16 volunteers, and a wild-type respiratory syncytial virus challenge 45 days later. Tyrrell and Reed showed that volunteers inoculated with influenza A virus during the incubation period of a rhinovirus were protected against influenza infection, although this did not occur during the incubation period of a coronavirus (22). The present study, also with a vaccine strain, failed to demonstrate heterologous interference between influenza A virus and parainfluenza type

1 virus given 7 days after influenza A virus infection. The mechanisms that permit heterologous interference to be detected in some of these experimental infections, but not others, remain unknown, but clearly dose and time of administration of challenge virus, and the type of primary virus infection (wild versus vaccine type) are three variables that need to be more fully evaluated. It is perhaps incorrect to assume that all live respiratory virus vaccines will induce heterologous interference, and this parameter of infection will have to be carefully evaluated with each new live virus vaccine.

The present investigation also presents data on the relationship between illness, quantitative virus shedding, and interferon response during experimental infection of adult volunteers with parainfluenza type 1 virus. In adults, the pattern of parainfluenza virus infection is similar to that of influenza A virus (17). The major difference in their patterns lies in the relationship between time of peak virus shedding to the time of the peak interferon response. For influenza A virus, the peak interferon response is detected 1 day after the day of peak virus shedding, whereas for parainfluenza virus, the peak interferon response is delayed until 3 days after the time of peak virus shedding. It would be more important to examine the quantitative relationships between level of illness, virus shedding, and interferon response in children undergoing the more serious lower respiratory tract illness observed during infections with parainfluenza viruses. If a similar delay in interferon response occurs in these children, then perhaps therapy with exogenous interferon or an antiviral drug could be considered.

ACKNOWLEDGMENTS

We thank Lula King for her excellent technical assistance; Louis Potash for providing and safety testing the parainfluenza type 1 virus; David Alling for help with statistical analysis; Superintendent Clarence L. Swain, Assistant Administrator for Programs John Johnson, and the other correctional officers at the Lorton Reformatory, Lorton, Va; and the resident volunteers for their help and cooperation, which made the study possible.

LITERATURE CITED

1. Baron, S., C. E. Buckler, R. M. Friedman, and R. V. McCloskey. 1966. Role of interferon during viremia. II. Protective action of circulating interferon. J. Immunol. **96**:17-24.
2. Bovarnick, M. R., J. C. Miller, and J. C. Snyder. 1950. The influence of certain salts, amino acids, sugars, and proteins on the stability of rickettsiae. J. Bacteriol **59**:509-522.
3. Byrne, E. B., B. J. Rosenstein, A. A. Jaworski, and R. A. Jaworski. 1967. A statewide mass measles immunization program. J. Am. Med. Assoc. **199**:619-623.
4. Cantell, K., H. Strandler, G. Hadhazy, and N. R. Nevonlinna. 1968. How much interferon can be prepared in human leucocyte suspensions, p. 223-232. *In* G. Rita (ed.), The interferons. Academic Press Inc., New York.
5. Cate, T. R., R. B. Couch, and K. M. Johnson. 1964. Studies with rhinoviruses in volunteers: production of illness, effect of naturally-acquired antibody and demonstration of a protective effect not associated with serum antibody. J. Clin. Invest. **43**:56-67.
6. Cate, T. R., R. G. Douglas, Jr., and R. B. Couch. 1969. Interferon and resistance to upper respiratory virus illness. Proc. Soc. Exp. Biol. Med. **131**:631-636.
7. Finter, N. B. 1967. Interferon in mice: protection against small doses of virus. J. Gen. Virol. **1**:395-397.
8. Fleet, W. F., R. B. Couch, T. R. Cate, and V. Knight. 1965. Homologous and heterologous resistance to rhinovirus common cold. Am. J. Epid. **82**:185-195.
9. Glezen, W. P., and F. W. Denny. 1973. Epidemiology of acute lower respiratory disease in children. N. Engl. J. Med. **288**:498-505.
10. Henle, W. 1958. Interference phenomena between animal viruses: a review. J. Immunol. **64**:203-236.
11. Hill, D. W., S. Baron, and R. M. Chanock. 1969. Sensitivity of common respiratory viruses to an interferon inducer in human cells. Lancet **2**:187-188.
12. Knight, V. 1964. The use of volunteers in medical virology. Prog. Med. Virol. **6**:1-26.
13. Matthews, J. H. J., S. E. Reed, and D. A. J. Tyrrell. 1974. The effect of prior inoculation with an enterovirus (LEV 4) on rhinovirus infection of volunteers. Arch. Gesamte Virusforsch. **45**:106-112.
14. Minor, T. E., E. C. Dick, J. A. Peterson, and D. E. Docherty. 1974. Failure of naturally acquired rhinovirus infections to produce temporal immunity to heterologous serotypes. Infect. Immun. **10**:1192-1193.
15. Murphy, B. R., S. Baron, E. G. Chalhub, C. P. Uhlendorf, and R. M. Chanock. 1973. Temperature-sensitive mutants of influenza virus. IV. Induction of interferon in the nasophanynx by wild-type and a temperature-sensitive recombinant virus. J. Infect. Dis. **128**:488-493.
16. Murphy, B. R., E. G. Chalhub, S. R. Nusinoff, and R. M. Chanock. 1972. Temperature-sensitive mutants of influenza virus. II. Attenuation of ts recombinants for man. J. Infect. Dis. **126**:170-178.
17. Murphy, B. R., E. G. Chalhub, S. R. Nusinoff, J. Kasel, and R. M. Chanock. 1973. Temperature-sensitive mutants of influenza A virus. III. Further characterization of the ts-1[E] influenza A recombinant (H3N2) virus in man. J. Infect. Dis. **128**:479-487.
18. Oie, H. K., C. E. Buckler, C. P. Uhlendorf, D. A. Hill, and S. Baron. 1972. Improved assays for a variety of interferons. Proc. Soc. Exp. Biol. Med. **140**:1178-1181.
19. Reichelderfer, T. E., R. M. Chanock, J. E. Craighead, R. J. Huebner, T. G. Ward, H. C. Turner, and W. D. James. 1958. Infection of human volunteers with type 2 hemadsorption virus. Science **128**:779-780.
20. Schlesinger, R. W. 1959. Interference between animal viruses, p. 157-189. *In* F. M. Burnet and W. M. Stanley (ed.), The viruses, vol III. Academic Press Inc., New York.
21. Smith, C. P., R. H. Purcell, J. A. Bellanti, and R. M. Chanock. 1966. Protective effect of antibody to parainfluenza type I virus in serum and nasal secretions. N. Engl. J. Med. **275**:1145-1152.
22. Tyrrell, D. A., and S. E. Reed. 1973. Some practical implications of interferon and interference, p. 438-442. *In* W. Braun and J. Ungar (ed.), Non-specific factors influencing host resistance, a re-examination. S., Karger, Basel.
23. Van Kirk, J. E., J. Mills V, and R. M. Chanock. 1971.

Evaluation of low temperature grown influenza A_2/Hong Kong virus in volunteers. Proc. Soc. Exp. Biol. Med. **136**:34-41.

24. Vella, P. P., R. E. Weibel, A. F. Woodhour, C. C. Mascoli, M. B. Leagus, O. L. Ittensohn, T. Stokes, Jr., and M. R. Hilleman. 1969. Respiratory virus vaccines. I. Field evaluation of trivalent parainfluenza virus vaccine among preschool children in families. 1967-1968, Am. Rev. Resp. Dis. **99**:526-541.
25. Wheelock, E. F., R. P. R. B. P. Larke, and N. L. Caroline. 1968. Interference in human viral infections: present status and prospects for the future. Prog. Med. Virol. **10**:286-347.