Interferon and Resistance to Upper Respiratory Virus Illness* (33941)

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Interferon has been detected during both experimental and natural virus infections of humans, and is considered a major factor in host defense against these agents (1–7). The present study describes the finding of interferon during infection with two additional respiratory viruses, rhinovirus type 15 (8) (NIH1734) and Coxsackie virus A type 21, and evaluates the role of interferon in the common cold syndrome caused by these viruses. Interferon appeared early enough during these virus infections to have had a role in causing recovery from illness, but no role for interferon could be demonstrated in resistance to rechallenge suggesting that other and perhaps more important nonspecific mechanisms of host defense against these illnesses remain to be defined.

Materials and Methods. Volunteers. Subjects were healthy adult males from several federal correctional institutions. Procedures by which they volunteered and were supervised have previously been described (9).

Virus inoculation, clinical, and virological procedures. Preparation of respiratory virus inocula, methods of administration of virus to volunteers, clinical evaluation, collection and testing of specimens for virus, and antibody assays have previously been described (10).

The schedule of inoculations is given in Table I. Four weeks after primary inoculation with rhinovirus, 12 of 14 volunteers were given a secondary inoculation with Coxsackie virus. At the time of the secondary inoculation, 9 additional men were given a primary inoculation with Coxsackie virus. All volunteers who received Coxsackie virus were free of detectable serum antibody to this virus at the time of inoculation including the 9 volunteers who received primary inoculation and the 12 who received a secondary inoculation. Two of the 14 volunteers who received rhinovirus possessed low levels of detectable serum antibody prior to challenge and these two men did not receive a secondary inoculation with Coxsackie virus (see Table II). All men became infected following each inoculation.

Interferon assay. Specimens tested for interferon were nasal washes (NW) and sera from 9 of the volunteers who received rhinovirus and from all 21 of the men who received Coxsackie virus (Table I). The NW were obtained as in previous studies (11), before inoculation, daily for the first week after inoculation, and 3 times weekly for an additional 2–3 weeks. The NW were irradiated in an open petri dish 6–7 cm from a germicidal lamp for four 5-min periods before assay for interferon. Sera collected daily during the period of maximum virus shedding and weekly thereafter were analyzed without any type of inactivation. All specimens were shown to be free of virus capable of producing cytopathic effect (CPE) at the time of testing.

Assay for interferon depended on inhibition of growth of Sindbis virus in human embryonic fibroblasts (HEF) similar to the method described by Wheelock and Sibley (3). Tissue culture tubes containing young HEF monolayers (WI-38) and 1.5 ml of medium[1] were inoculated with 0.2 ml of serial 4-fold dilutions of the specimen beginning with 1:4, 2 tubes/dilution. Tubes were incubated at 34° for 18–24 hr on a drum rotating 12 times /hr. Medium was then removed and

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[1] Equal parts of medium no. 199, and Eagle's medium no. 2, 2% heat-inactivated calf serum, 0.4% saturated solution of bicarbonate, and antibiotic.

TABLE I. Schedule of Inoculations.

Virus inoculum	Method given	Dose ($TCID_{50}$)[b]	No. of volunteers having: Primary inoculation	Secondary inoculation	Specimens tested for interferon
Rhinovirus type 15, HEF_2[a]	Inhalation of aerosol	26–71	8	—	5
	Intranasal instillation	45	6	—	4
Coxsackie A type 21, strain 48654, HEF_2	Inhalation of aerosol	260	6	6	12
	Intranasal instillation	35	3	6	9

[a] Passaged twice in human embryonic fibroblasts.
[b] Fifty percent tissue culture infective doses.

1.4 ml of fresh medium containing 10–16 $TCID_{50}$ of Sindbis virus was added to each tube. When CPE involved 50–75% of the HEF monolayer in each of 8 control tubes inoculated with the test dose of virus, the assay for Sindbis inhibitor (interferon) in the specimens was read. Greater than 50% reduction in the amount of CPE was considered significant, and the titer per 0.2 ml of specimen was estimated by the Kaerber method. Results from repeated assay of 89 specimens collected during the period of maximum virus shedding revealed no more than 2-fold variation in 82 (92%) of the pairs of Sindbis inhibitor titers, and 4-fold variation in 7 (8%) pairs.

TABLE II. Illness Severities and Highest Titers of Interferon in Nasal Wash during Respiratory Virus Infections.

Primary rhinovirus infection		Secondary Coxsackie virus infection		Primary Coxsackie virus infection	
Illness[a]	Interferon titer	Illness	Interferon titer	Illness	Interferon titer
0	<4	++	<4	0	<4
0	4	++	4	+	8
0	—[b]	++	4	+	8
0	—	+	16	+	32
+	—	++	8	++	<4
+	<4	+	<4	++	16
+	—	+	4	++	16
+	—	0	<4	++	32
+	8	0	<4	++	32
+	<4	0[c]	8		
+	8				
++	8	+	8		
++	8	+	<4		
++	<4				

[a] 0 = no respiratory illness; + = afebrile respiratory illness; ++ = febrile respiratory illness (equal to or greater than 37.6° orally and/or 38.1° rectally).
[b] Not done.
[c] Following inoculation with Coxsackie virus this volunteer developed fever (38.5° rectally) with a furuncle, but he had no respiratory symptoms and is not included as being ill due to the virus.

The Sindbis virus used in these studies was the prototype AR339 strain (Taylor)[2] and all studies to be reported were performed with a pool of virus prepared in HEF. Duplicate assays for interferon were performed on specimens from 12 volunteers using a Sindbis virus pool prepared from infected suckling mice, and results were comparable to those with the pool prepared in HEF.

Treatment of Sindbis inhibitor at pH2 and trypsin digestion were performed as previously described (11). Neutralization of Sindbis inhibitor in NW with hyperimmune guinea pig antiserum against the infecting respiratory virus was tested for by incubating the specimens with equal portions of antiserum at room temperature for 5 hr prior to inoculation of tissue cultures.

Results. Severities of illness and highest titers of interferon appearing in NW during infection with rhinovirus type 15 and Coxsackie virus A type 21 are listed in Table II. The 12 volunteers who received both primary rhinovirus and secondary Coxsackie virus inoculations (columns 1–4) are arranged according to increasing severity of illness in column 1. The last and fourth from last men represented in columns 1 and 2 received only primary rhinovirus inoculation and possessed antibody against that virus before inoculation. The 9 volunteers who received primary Coxsackie virus inoculation (columns 5 and 6) are arranged according to increasing illness severity in column 5. (None of the sera contained detectable interferon.)

Comparing the two primary inoculation groups in Table II, both the frequency of respiratory illness and the severity as measured by occurrence of fever were less during rhinovirus infection than during infection with Coxsackie virus (column 1 versus column 5). Similarly, the frequency of detection of interferon and the titers detected were less during rhinovirus infection (column 2 versus column 6).

Illnesses following secondary inoculation with Coxsackie virus were also less frequent and milder than those following primary inoculation with this virus (column 3 vs. column 5), though the differences are not statistically significant. In addition, duration of illness in the secondary inoculation group tended to be shorter than that in the primary group; mean durations were 2.66 and 3.00 days, respectively.

Marked differences in the amount of interferon produced by the two groups of Coxsackie virus volunteers were noted. After consecutive NW specimens from each volunteer were assayed in several different tests, specimens containing interferon as well as others collected at the appropriate time from each man were re-assayed in a single test. Comparison of peak interferon titers from this test indicates that the two groups were different (column 4 vs. column 6, p less than 5%, Wilcoxon test, two-tailed) (12), with values from the secondary group being lower. (The route of inoculation did not influence this difference.)

Although responses to secondary inoculation with Coxsackie virus more closely resembled those following primary rhinovirus inoculation than those of the primary Coxsackie virus group, all evidence indicated that the second infection was with Coxsackie virus only. The volunteers had ceased shedding rhinovirus approximately 2 weeks prior to their secondary Coxsackie virus inoculation, and were free of symptoms and signs of respiratory illness. In addition, the first and last isolates from each man in the secondary group were identified as Coxsackie virus A type 21, and each volunteer developed a significant titer of serum antibody to Coxsackie virus A type 21, 3–4 weeks following the secondary inoculation.

However, the severity of rhinovirus illness appeared to influence the severity of secondary Coxsackie virus illness. Illness severities during the two infections (columns 1 and 3, Table II) showed a negative correlation (Spearman's rank correlation with correction for ties, $r = -.506$, p less than 5%, one-tailed) (12), *i.e.*, the greater the severity of rhinovirus illness, the milder the illness with Coxsackie virus infection. This negative correlation remains statistically significant if the tenth volunteer is excluded.

[2] Kindly provided by Dr. N. H. Wiebenga.

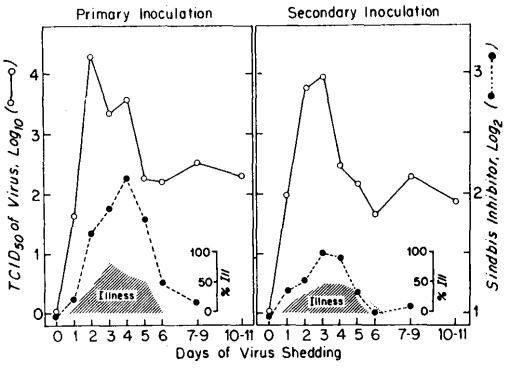

FIG. 1. Mean titers of virus and interferon (Sindbis inhibitor) in nasal wash during infection and illness due to Coxsackie virus A type 21. Titers are per 0.2 ml of NW. For Sinbis inhibitor, a titer of 1 indicates none detected. The size of the shaded area is proportional to the daily incidence of illness.

Results of interferon assays during both primary rhinovirus and secondary Coxsackie virus infections are available for seven volunteers. No correlation exists between the two peak interferon titers which appear in columns 2 and 4, Table II.

In Fig. 1, mean titers of virus and of interferon are shown along with the period of illness for the volunteers infected with Coxsackie virus. Virus was characteristically first detected in specimens collected 1–3 days following inoculation, and the data from each man are aligned according to this first day of virus shedding. Among the primary inoculation group (left panel), the amount of virus in NW rapidly reached a peak on the second day of shedding and then decreased to a low titer which persisted for an additional 1–2 weeks. Interferon first appeared in NW with the onset of virus shedding and characteristically reached peak titer for individual volunteers on either the same day or 1–2 days after peak titers of virus occurred. Interferon then disappeared. In preliminary studies of volunteers infected with rhinovirus type 15, interferon was not detected beyond day 9 even though low titers of virus persisted for several additional days. Respiratory illness was associated with the period of maximum virus shedding. As the interferon titer approached its peak, there was a decrease in both the incidence of illness and the amount of virus shed.

Timing of events following secondary inoculation (right panel, Fig. 1) was similar to that following primary inoculation. Reductions in frequency and duration of illness and in frequency of detection and titer of interferon have been described. In addition, volunteers of the secondary group tended to shed smaller amounts of virus; comparison of peak virus titers for individual volunteers indicates that the probability that the two groups were different is between 5 and 10% (Wilcoxon test, two-tailed).

Properties of Sindbis inhibitor were examined for specimens of high titer. Three changes of media before addition of Sindbis virus to HEF tubes previously incubated with dilutions of a specimen produced no change in titer. The mean titer of three specimens treated with heat (56°, 30 min) was identical to that after treatment of the specimens with ultraviolet light alone. Treatment of specimens at pH 2 failed to reduce titers; however, treatment with trypsin resulted in reduction of inhibitor to below detectable levels. In addition, Sindbis inhibitor in three specimens was not inactivated by hyperimmune antiserum against the inducing virus, Coxsackie virus A type 21.

Discussion. Release of interferon into respiratory secretions during infection with a rhinovirus and during infection with Coxsackie virus A type 21 is described for the first time. Similar results have been reported by others during infection with influenza viruses (4, 7). Also, interferon has been found in serum by others during influenza (7), during respiratory illnesses of unknown etiology (3), and during virus infections with recognized viremia (5, 6). The failure to detect interferon in serum of the present volunteers may relate to the lack of a demonstrable viremia (13, 14).

That the material being measured as Sindbis inhibitor was actually interferon is suggested by the transient appearance of the substance in nasal wash specimens at the time of or just after peak virus shedding. Also, the properties of Sindbis inhibitor included inability to wash the inhibitory effect

from previously exposed cells, stability to heat (56°, 30 min) and acid (pH 2), failure to be inactivated by antiserum against the inducing virus, and inactivation by trypsin; these properties are compatible with those described for interferon (1).

In an earlier report, heterologous resistance to respiratory virus illness lasting at least 5, but not 16 weeks after an initial infection was described using rhinoviruses of different serotype, and epidemiologic data compatible with a similar period of nonspecific resistance following naturally-occurring colds was reviewed (15). In the present study, resistance to respiratory virus illness 4 weeks after an initial illness due to an entirely different virus was found; the degree of resistance was inversely related to the illness severity during the initial infection, *i.e.*, the greater the severity of illness during primary rhinovirus infection, the greater the protection afforded against illness during secondary Coxsackie virus infection. The role of interferon in this phenomenon was investigated.

Interferon appeared early enough in nasal wash specimens from members of each group of volunteers in the present study to have had a role in causing a decrease in virus replication and recovery from illness. However, the amount of interferon detected during secondary Coxsackie virus infection was considerably less than that found during primary Coxsackie virus infection in other volunteers, and the titers of interferon found during primary rhinovirus and secondary Coxsackie virus infections for individual volunteers bore no relationship to each other. Thus, interferon induced by preceding rhinovirus infection did not appear to lead to enhanced production of interferon during secondary Coxsackie virus infection with suppression of the latter infection, a suggested mechanism of protection for some virus infections (16).

Cellular resistance induced by interferon from the primary rhinovirus infection might explain the findings in the secondary group. However, *in vitro* studies suggest that interferon mediated cellular resistance would not have lasted for a period as long as that between rhinovirus and Coxsackie virus infections in the absence of continued production of interferon (17); the latter could not be detected. Prolonged *in vivo* resistance to virus infection has been reported to follow injection of the interferon inducer, pyran, but it is not clear that the prolonged resistance is due to the briefly demonstrable interferon production (18). In another *in vivo* model presumably mediated by interferon, the resistance of alveolar monocytes to rabbitpox virus following intratracheal injection of parainfluenza-3 virus was undetectable after 4–5 days (19), and this interval is too short to explain the findings of the present study.

These results suggest that factors other than interferon may account for the temporary, nonspecific resistance against respiratory virus illness following an initial infection as defined in this and earlier studies, and that important mechanisms of host defense against these illnesses have yet to be defined.

Summary. The role of interferon in the common cold syndrome caused by Coxsackie virus A type 21 and by rhinovirus type 15 was evaluated in volunteers. Interferon appeared in nasal wash transiently and early enough during infection to have had a role in causing recovery from illness; it was not found in serum. The influence of one respiratory virus infection on a subsequent one was evaluated by challenging men with Coxsackie virus 4 weeks after a primary rhinovirus infection. Increasing severity of illness during primary rhinovirus infection was associated with decreasing severity of illness during secondary Coxsackie virus infection. No correlation existed between interferon titers in nasal wash during the two infections, but titers during secondary Coxsackie virus infection were less than those during primary Coxsackie virus infection in other volunteers. Interferon did not appear to account for the resistance to Coxsackie virus illness afforded by a preceding rhinovirus common cold.

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