

Intranasal Interferon as Protection Against Experimental Respiratory Coronavirus Infection in Volunteers

P. G. HIGGINS,^{1*} R. J. PHILLPOTTS,¹ G. M. SCOTT,¹ J. WALLACE,¹ L. L. BERNHARDT,² AND D. A. J. TYRRELL¹

Medical Research Council Common Cold Unit, Harvard Hospital, Salisbury, Wiltshire SP2 8BW, England,¹ and Hoffmann-La Roche Inc., Nutley, New Jersey 07110²

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In a double-blind, placebo-controlled study, self-administered intranasal human interferon α A produced by recombinant DNA technology was given both before and after virus challenge with a respiratory coronavirus. The incidence of colds, the severity of symptoms and signs, and virus replication were all reduced in subjects receiving interferon as compared with those given placebo.

Interferon given as a nasal spray prevents clinical illness and reduces the incidence of infection in volunteers exposed to several different rhinovirus serotypes. These results have been shown to be true for interferon derived from human leukocytes and partially purified (2), for similar material highly purified by immunoabsorption chromatography with monoclonal antibody (6), and for interferon produced in *Escherichia coli* as a result of genetic engineering (5).

Rhinoviruses are the most frequent cause of the common cold, but infection with many other viruses contributes to the frequency with which this syndrome occurs. To be effective in preventing colds, interferon must be as efficient against at least some of these other agents as it has been shown to be against rhinoviruses. Probably the second most common cause of colds is infection with one of the respiratory coronaviruses, which differ markedly from the rhinoviruses. Therefore, the present study was undertaken to determine whether another preparation of human interferon α produced by recombinant DNA techniques could protect volunteers from symptomatic infection with a human respiratory coronavirus.

MATERIALS AND METHODS

Virus. The LP strain of coronavirus 229E was used, contained in a filtered nasal wash. Between 89 and 407 50% tissue culture infective doses of virus, as determined by back titration of the inoculum, were instilled into the nose of each volunteer challenged with the virus.

Interferon and placebo. Freeze-dried recombinant human leukocyte interferon α A (Hoffmann-La Roche) in buffered human albumin was reconstituted with sterile distilled water to a concentration of 10^7 U/ml. Fresh material was prepared every other day and

stored at 4°C. An identical preparation containing the excipient but without interferon was used as the placebo.

Volunteers. Normal healthy adults between the ages of 18 and 50 years were screened for hematological and biochemical abnormalities before and at the end of medication. The volunteers were isolated in flats in groups of two or three at the Common Cold Unit, Salisbury, England.

Plan of the experiment. This study was approved by the Ethical Committee at Northwick Park Hospital, Harrow, England. Volunteers were matched for age and sex and were assigned to receive either interferon or placebo in such a way that only one preparation was used in any one flat. After a 2-day quarantine period, the volunteers administered interferon or placebo to themselves, intranasally, three times a day for 4 days by a finger-actuated spray (Mistette Mark II, kindly supplied by Calmar-Albert, GmbH, Hemer, Federal Republic of Germany). Each medication, two sprays of approximately 0.1 ml each to each nostril, was supervised by a member of the staff. The intended dose of interferon was, therefore, 4×10^6 U, and the total dosage over 4 days was 4.8×10^7 U. The actual amount of medication used was determined by subtracting the weight of the sprays at the end of treatment from their initial weight. Six volunteers given interferon and seven given placebo were inoculated with balanced salt solution to ensure the double-blind status of volunteers and the clinical observer. The remaining volunteers were challenged with virus 4 h after the fourth dose of medication. Neither the volunteers nor the clinical observer was aware of which preparation, interferon or placebo, virus or saline, the subject was given. Each volunteer was assessed daily and assigned a score on the basis of the presence and severity of symptoms and clinical signs of a cold (1). The total number of tissues used by each volunteer was recorded, as was the weight of the nasal secretion they contained. The severity of the colds which occurred was assessed by our usual procedures (1).

The presence of virus was sought in the nasal washings obtained from each volunteer on days 2 to 6 after virus challenge. Undiluted nasal wash (0.2 ml)

was inoculated into cultures of the C-16 line derived from MRC-C cells (3) in the presence of calf antilymphoblastoid interferon (5), kindly supplied by K. Fantes. At least one isolate from each volunteer was shown to be 229E virus by neutralization with specific rabbit antiserum. Antibody to LP virus was measured by a microneutralization test (4) in C-16 cells. Two samples of serum were assayed, one collected at the commencement of the trial and another obtained 2 or 3 weeks after virus challenge; a fourfold or greater rise in antibody titer was considered evidence of infection.

Statistical analysis. Differences in the frequency of colds, virus isolations, and rises in antibody titer between the interferon and placebo groups were tested for significance by the X^2 test with Yates' correction. The clinical score and nasal secretion weight were evaluated by the Mann-Whitney U test.

RESULTS

Eighty-three volunteers took part in the experiment, which was spread over four trial periods. As there was no statistically significant difference in the incidence of colds among the four periods, it was considered justified to treat all four trials as one experiment. The control volunteers who received balanced salt solution in place of virus tolerated both interferon and placebo equally well. The highest total clinical score achieved by any of these volunteers receiving interferon was 1 (equivalent to the presence of one sign of a cold of mild intensity on 1 day). The remaining 70 volunteers, all of whom were challenged with virus, were found to be equally divided between those given interferon and those given placebo. Similarly, the ages, sexes and pretrial antibody titers of the volunteers in the two groups were seen to be well matched (Table 1). The mean total amount of

interferon actually received, as determined by the difference in weight of the sprays before and after use, was 3.53×10^7 U. Comparison of the results of hematological and biochemical tests on sera obtained before and after medication showed no significant changes in values in either the interferon or placebo group.

The clinical response to virus challenge of these two groups and the virological findings are summarized in Table 1. Mild or worse colds were considered significant, as it was always possible to find laboratory evidence of infection: either virus was isolated or a fourfold-or-greater rise in antibody titer was demonstrated or both. In addition, such illnesses did not occur in volunteers challenged with saline. Two significant colds occurred among the 35 volunteers who received interferon, whereas 13 colds were observed in the 35 volunteers given placebo ($P < 0.01$). Both colds in the interferon group occurred among the six volunteers with pretrial antibody titers of $<1:2$, as compared with four colds among the 10 volunteers in the comparable placebo group. The mean daily clinical scores and nasal secretion weights are significantly greater for volunteers receiving placebo than for those receiving interferon on days 3, 4, and 5 and days 4 and 5 after inoculation, respectively (Fig. 1). The proportion of volunteers excreting virus was significantly lower on all days after inoculation for those receiving interferon than for those receiving placebo ($P < 0.0001$). Furthermore, a higher proportion of volunteers receiving placebo showed a fourfold-or-greater increase in serum antibody titer to the challenge virus than did those on interferon ($P < 0.05$).

TABLE 1. Clinical and virological findings in volunteers challenged with 229E-like virus

Volunteer group and pretrial antibody titer (no. of volunteers)	No. with clinically diagnosed colds		Virological findings		
	Mild or worse ^a	Very mild or absent	Rises in antibody titer ^b	Virus isolated ^c	Either or both ^c
Interferon^d					
<2 (6)	2	4	4 ^e	5	5
2-8 (15)	0	15	5	5	5
>8 (14)	0	14	0	2	2
Placebo^f					
<2 (10)	4	6	10	10	10
2-8 (10)	4	6	7	9	9
>8 (15)	5	10	3	11	11

^a $P < 0.01$.

^b $P < 0.05$.

^c $P < 0.001$.

^d Fourteen males, 21 females; mean age, 31.6 ± 10.1 years.

^e Convalescent serum was not received from one volunteer.

^f Fourteen males, 21 females; mean age, 30.8 ± 10.7 years.

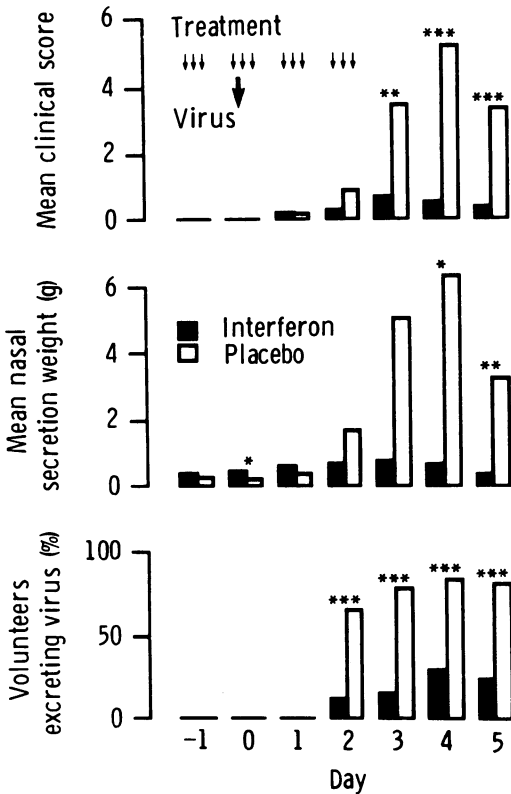


FIG. 1. Mean clinical scores, nasal secretion weights, and virus excretion for volunteers receiving human interferon αA or placebo. Symbols: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Not significant, $P \geq 0.05$.

DISCUSSION

This study has shown that interferon given intranasally before and after virus challenge has a marked protective effect against experimental infection of volunteers with a respiratory coronavirus. Not only does interferon reduce the frequency and severity of clinical symptoms, but it also reduces the degree of virus replication. The fact that there was no significant difference in the incidence of colds between those receiving interferon and those given placebo in cases where the pretrial antibody titer was $<1:2$ is compatible with the hypothesis that interferon and antibody work synergistically. However, the numbers involved are too small to prove this hypothesis, and our experience with larger numbers of volunteers given human interferon α and challenged with rhinovirus showed that similar proportions of those with and without detectable antibody were protected by interferon. Of the 35 volunteers receiving interferon, 12 showed evidence of infection, although only 2 suffered from

clinically diagnosed colds. This result would indicate that not only can interferon protect against clinical illness, but it also can allow a proportion of the exposed subjects to develop natural immunity.

In this experiment, the amount of interferon given and the frequency of administration were based on the results of dose-ranging studies with recombinant interferon $\alpha 2$ used to protect volunteers from infection with rhinoviruses (3a). The total amount of interferon used in the study was less than half of that shown previously to protect against infection with rhinovirus type 9 (6), and it may well be that experimental infection in volunteers is a more stringent test for the drug than naturally acquired infections and that the total dosage can be reduced still further and continue to be effective.

Although the prevention of upper respiratory tract infections is important, especially for those in whom infection may progress to more severe illness (e.g., chronic bronchitics or asthmatics), it commits the subject to long-term exposure to intranasal interferon, which may be undesirable. It would be very much more satisfactory if interferon could be used as a treatment, and experiments designed to demonstrate a therapeutic effect are now being undertaken.

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LITERATURE CITED

1. Beare, A. S., and S. E. Reed. 1977. The study of antiviral compounds in volunteers, p. 27-55. In J. Oxford (ed.), *Chemoprophylaxis and virus infections of the respiratory tract*, vol. 2. CRC Press, Inc., Cleveland.
2. Merigan, T. C., S. E. Reed, T. S. Hall, and D. A. J. Tyrrell. 1973. Inhibition of respiratory virus infection by locally applied interferon. *Lancet* i:563-567.
3. Philippotts, R. J. 1983. Clones of MRC-C cells may be superior to the parent line for the culture of 229E-like strains of human respiratory coronavirus. *J. Virol. Methods* 6:267-269.
- 3a. Philippotts, R. J., G. M. Scott, P. G. Higgins, J. Wallace, D. A. J. Tyrrell, and C. L. Gauci. 1983. An effective dosage regimen for prophylaxis against rhinovirus infection by intranasal administration of HuIFN- α_2 . *Antiviral Res.* 3:121-136.
4. Philippotts, R. J., J. Wallace, D. A. J. Tyrrell, D. S. Free-stone, and W. M. Shepherd. 1983. Failure of oral 4,6-dichloroflavan to protect against rhinovirus infection in man. *Arch. Virol.* 75:115-121.
5. Scott, G. M., R. J. Philippotts, J. Wallace, C. L. Gauci, J. Greiner, and D. A. J. Tyrrell. 1982. Prevention of rhinovirus colds by human interferon alpha-2 from *Escherichia coli*. *Lancet* ii:186-187.
6. Scott, G. M., R. J. Philippotts, J. Wallace, D. S. Secher, K. Cantell, and D. A. J. Tyrrell. 1982. Purified interferon as protection against rhinovirus infection. *Br. Med. J.* 284:1822-1825.