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A study of intranasally administered interferon A* (rIFN-α2A) for the seasonal prophylaxis of natural viral infections of the upper respiratory tract in healthy volunteers

G. A. TANNOCK, S. M. GILLETT, R. S. GILLETT, R. D. BARRY, M. J. HENSLEY, R. HERD, A. L. A. REID AND N. A. SAUNDERS

Faculty of Medicine, The University of Newcastle, David Maddison Clinical Sciences Building, Royal Newcastle Hospital, Newcastle, New South Wales, 2300, Australia

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SUMMARY

The efficacy of interferon A (rIFN-α2A), an *Escherichia coli*-derived interferon, in the prophylaxis of acute upper respiratory tract infection, was evaluated in a community-based double-blind placebo-controlled study in the Australian winter of 1985. The trial population of 412 healthy volunteers (190 males and 222 females, aged 18–65 years) self-administered 1·5, 3·0 and 6·0 megaunits (MU) of interferon A per day or a placebo, intranasally for 28 days.

The period of study coincided with an outbreak of H3N2 influenza A (detected in 35 of the 107 acute specimens) as well as substantial numbers of respiratory syncytial virus and adenovirus infections. Rhinoviruses were isolated from only three specimens. In many cases, subjects had laboratory and clinical evidence of having had more than one respiratory tract infection during the period of the study. Viruses were detected in 54 or 107 acute specimens (49%).

No statistically significant differences were noted between the various treatment groups in the incidence of laboratory-proven viral infection (virus isolation and/or antibody response). Analysis of reported symptoms indicated that blood-tinged mucus and nasal stuffiness occurred more frequently with higher doses of interferon. There appeared to be no clinical benefit from the use of interferon A in the amelioration of symptoms.

INTRODUCTION

The biological role of interferons as inhibitors of viral multiplication has been known for 30 years (Burke, 1985). Although several earlier studies using crude preparations suggested a therapeutic role for interferon in the prophylaxis of viral respiratory infections (Merigan *et al.* 1973; Greenberg *et al.* 1978) definitive clinical studies have only been possible in the past 5 years, with the advent of defined interferons produced by recombinant DNA techniques.

There have been a number of reports on the use of recombinant DNA interferon

* Roferal-A™, F. Hoffman–La Roche, Basle, Switzerland.

in the prevention of respiratory infections (Scott *et al.* 1982; Hayden & Gwaltney, 1983; Farr *et al.* 1984). High doses (10 MU per day) were shown to exhibit infections by rhinoviruses and coronaviruses but prolonged administration resulted in unacceptable side-effects which included nasal irritation, mucosal ulceration and nasal bleeding (Merigan *et al.* 1973; Hayden, Gwaltney & Johns, 1985; Burke, 1985). A trial of interferon low dose (1 megaunit per day) revealed no identifiable clinical benefit (Samo *et al.* 1984). Considerable prophylactic efficacy against laboratory-documented rhinovirus infection was noted in studies where family members were administered either 1·5 MU per day for 5 days or 5 MU per day for 7 days after one member of the family experienced symptoms (Herzog *et al.* 1986; Douglas *et al.* 1986; Hayden *et al.* 1986).

This paper reports the results of a 28-day, placebo-controlled prospective study of the efficacy of intra-nasally administered interferon-α2a (Roferal–Roche) carried out in Newcastle, New South Wales, Australia, during the winter of June–July, 1985.

MATERIAL AND METHODS

Selection of subjects and sample size

The trial population comprised 190 males and 222 females between the ages of 18 and 65 years, who volunteered from the local community in response to advertisements in doctors' surgeries and the media. Exclusion criteria included: inadequate contraception; a history of allergies or conditions of the respiratory tract likely to interfere with the evaluation of the treatment drug; a history of frequent nose bleeds; regular use of certain medications including aspirin and/or anti-inflammatory agents; and the occurrence of respiratory illness within 2 weeks of trial commencement. The use of intranasal medications or eyedrops of any sort was not permitted during the trial. Eligibility was determined by interview and examination by a physician who collected blood and urine samples for laboratory examination 2 weeks before the start of trial medication.

Pre- and post-trial questionnaires were completed to provide the following data: demographic details; overall symptoms; factors known to influence the incidence or severity of viral infections, including the number of other adults in the study and children in each household, past history of respiratory illness and smoking habits; acceptability of the treatment.

The size of the study population was calculated to be large enough to detect, with $\alpha = 0{\cdot}05$ and $\beta = 0{\cdot}20$, a 50% reduction in the incidence of clinically detected and laboratory-documented respiratory infections between the placebo and each treatment group, assuming a 40% incidence of infection in the placebo group and between the placebo and all treatment groups combined, assuming that specific infection could be identified in 30% of the placebo group.

Trial medication

Subjects administered a daily dose of 1·5, 3·0 and 6·0 MU of interferon A or a placebo (human serum albumin 5 mg/ml, sodium chloride 9 mg/ml and benzalkonium chloride 0·2 mg/ml) for 28 days, by intranasal spray twice daily. The interferon A was *Escherichia coli*-derived, recombinant interferon (Roferal-A™), provided under code by F. Hoffman–La Roche, Basle, Switzerland in containers

that were numbered serially and allocated randomly in blocks of 20. The upper and lower levels of interferon dosage were chosen in the expectation that the protective efficacy against susceptible viruses (e.g. rhinoviruses) would be > 90% and > 50% respectively.

Outcome measurements

Outcome variables included symptoms of acute upper and lower respiratory tract infection, previously documented side-effects of interferon (nasal bleeding, mucosal ulceration and stuffiness), virus isolation in acute specimens and seroconversion over the trial period in all subjects.

Symptoms were recorded using a severity scale of 0–3 for 35 days. The fifth week was a post-trial observation period. The weekly symptom cards were monitored by trial nurses in weekly visits to clinics. In the event of respiratory illness, volunteers were required to contact the trial nurse for collection of nasal and throat washings for viral isolation, and acute and convalescent blood samples for serological identification. A further examination with laboratory tests was conducted at the conclusion of the trial (day 29) or when the subject reported symptoms characteristic of the side-effects of interferon.

Discrimination between symptoms of acute upper respiratory tract illness and nasal side-effects was a potential difficulty and hence the symptom data were analysed using a retrospectively developed symptom complex algorithm. Symptoms of blood-tinged mucus and stuffy nose were excluded from the algorithm, since preliminary analysis had indicated they were related to interferon dose and hence likely to be side-effects.

Defined symptoms for upper respiratory tract episodes (URTE) were dry nose, runny nose, sneezing, sore throat, cough, hoarseness/irritated throat, fever and chills. For generalized influenza episodes (GIE), additional symptoms were sore eyes, headache, muscular ache, nausea and diarrhoea. URTE and GIE were not regarded as mutually exclusive categories. The ratings for all symptoms were added to produce a daily total symptom score (DTSS). Any DTSS above zero for a particular day was added to that for the next 2 days. If the combined score was greater than or equal to 4 for URTE symptoms or 6 for GIE symptoms, an episode was considered to have started. An episode was considered to have ended if a DTSS of 0 was reported for a particular day followed by one of < 2 on the next day.

Laboratory procedures

Throat swabs for the detection of bacterial pathogens were obtained immediately before the medication phase of the trial began and after its completion, and whenever acute samples were collected. Haematological and biochemical profiles and urinalysis were obtained for each participant at pre- and post-trial examinations.

Immunofluorescence of nasal washings was used to detect respiratory syncytial virus, influenza A and B, parainfluenza 1 and 3 and adenoviruses. For attempted virus isolation, aliquots consisting of 0·1 ml of nasal and throat washings were inoculated to cell cultures of primary Cynomolgus monkey kidney epithelial cells and the BSC-1, Hep-2, HeLa (rhinovirus sensitive) and MRC-5 lines. After

adsorption for 30 min, 1 ml of maintenance medium containing inhibitory concentrations of polyclonal or monoclonal anti-interferon sera were added to each culture. The cultures were rolled at 0·5 r.p.m. at 34 °C and examined microscopically for cytopathic effects (CPEs) on alternative days. When a CPE was noted, the culture was retained for characterization. Negative cultures were passaged up to three times and then discarded.

Two techniques were used for viral serology on pre-trial, post-trial and acute and convalescent serum samples: haemagglutination inhibition tests for influenza A/Philippines/2/82 (H3N2), influenza A/Victoria/3/85 (H3N2), influenza A/Chile/1/83 (H1N1), influenza B/USSR/100/83, parainfluenza, 1, 2 and 3 and human coronavirus OC43; complement fixation tests for adenoviruses, respiratory syncytial virus, and *Mycoplasma pneumoniae*. A fourfold or greater rise in titre was the criterion for an antibody response.

Statistical methods

For discrete data, such as the number of episodes, virus isolations and questionnaire responses, contingency tables were constructed and Chi-squared statistics calculated. Average daily reported symptoms (the average daily reported score per person for each symptom) were analysed using the non-parametric Kruskal–Wallis test (Dixon *et al.* 1985). Some linear modelling was undertaken to assess the impact of time and dose on the side effect of blood-tinged mucus, using the generalized linear interacting modelling system (GLIM; Nelder & Wedderburn, 1972) and incorporating a standard binomial error. For regression analysis, the dose was transformed by taking the natural logarithm of dose units plus one (Zarr, 1974). The independent variable was used before and after probit transformation (Coulton, 1974) and the best fit reported. All calculations were done using the ABSTAT software package on a microcomputer. Relative risk with 95% confidence intervals was calculated for the main outcome measures (Armitage & Berry, 1977). Except where otherwise stated, the 5% level of significance was used.

RESULTS

The age and sex of subjects are set out in Table 1 according to the treatment schedules. More females were enrolled than males but similar proportions were present in all but one treatment group. The exception was the group receiving 3 MU per day in which there were more males than females. However, the difference was not statistically significant.

Participants included students, nurses, unemployed and retired persons within the Newcastle-Lake Macquarie region of New South Wales. Analysis of the pre-trial questionnaire indicated that the groups were reasonably homogeneous with respect to clinically relevant factors. No differences were found between the groups in smoking habits, prior history of respiratory illness, influenza immunization, medication and alcohol consumption. Differences between the groups were: a reduced number of adults in the households of male participants receiving the 3 megaunit dosage with an average of 1·3 compared with 1·5, 1·6 and 1·5 for the other groups ($\chi^2_9 = 20{\cdot}521$; $P = 0{\cdot}015$), a reduced number of children and adolescents in the male placebo and 1·5 MU groups with averages of 0·8 and

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Table 1. *Age and sex of subjects by treatment group*

Treatment group	Placebo ($n = 102$)	1·5 MU/day ($n = 104$)	3·0 MU/day ($n = 103$)	6·0 MU/day ($n = 103$)	Total ($n = 412$)
Males	45	47	56	42	190
Females	57	57	47	61	222
Proportion of males	0·44	0·45	0·54	0·41	0·46
Average age (years) (±S.D.)	32·5 ±12·4	32·5 ±12·2	33·9 ±11·7	33·2 ±12·4	33·0 ±12·1

Table 2. *Distribution of illness indicators among treatment groups*

Treatment group	Placebo	1·5 MU/day	3·0 MU/day	6·0 MU/day	Total
URTE (1 or more)	33	32	39	39	143
Av. duration (days)	6	8	8	6	7
GIE (1 or more)	33	27	38	30	128
Av. duration (days)	6	10	10	8	8
Acute samples taken	30	22	28	27	107
Nasal erosions	11	17	13	23	64
No. with > 1 symptom	90	99	95	98	382
Withdrawals	3	3	7	2	15

URTE, upper respiratory tract episodes.
GIE, general influenza episodes.
1 or more, implies that the individuals had one or more episodes.

0·6 respectively, compared with 1·1 and 1·0 for the other groups; ($\chi^2_6 = 15{\cdot}143$; $P = 0{\cdot}019$); a higher percentage of 'vitamin users' in the 6 MU group (55% compared with 38%, 41 and 40% for the other groups; $\chi^2_3 = 8{\cdot}057$; $P = 0{\cdot}04$).

No trends were noted in the haematology tests or liver enzyme profiles obtained from participants before and after the study and when a subject withdrew from the study.

The distribution of URTE, GIE and nasal erosions as well as the numbers of acute samples taken and subjects who withdrew among the various treatment groups is shown in Table 2. The illness data determined by the algorithm described in the Methods section cover the period from 72 h after medication started to 24 h after it ceased.

The figures represent the number of individuals in each group who suffered one or more URTE or GIE. Smaller proportions of subjects reported for sampling from the treatment groups compared to the placebo group but the differences were not statistically significant. Hyperaemic nasal mucosa with or without erosions was the only abnormality noted from the post-trial clinical examination and laboratory tests. All erosions were healed within 14 days of the completion of medication.

Fifteen subjects were withdrawn from the study before the completion of medication. Ten were excluded for violation of the protocol after being placed on antibiotics for a range of infections. Three withdrew because of the following reasons: severe headaches – 1·5 MU group; inflamed nasal mucosa and swollen face, which was considered to be allergic nasal reaction – 3 MU group; headache,

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Table 3. *Weekly prevalence of reported blood-tinged mucus*

	Placebo	1·5 MU/day	3·0 MU/day	6·0 MU/day
Week 1	10 (9·8%)	17 (16·3%)	14 (13·6%)	21 (20·4%)
2	5 (5·0%)	13 (12·5%)	21 (21·2%)	25 (24·8%)
3	5 (5·0%)	17 (16·5%)	24 (24·5%)	30 (29·7%)
4	9 (9·1%)	21 (20·6%)	27 (28·1%)	33 (32·7%)
5	5 (5·1%)	17 (16·8%)	22 (22·9%)	29 (28·7%)
Average	(6·8%)	(16·5%)	(22·0%)	(27·2%)
Group no. (average)	100	103	98	101

Table 4. *Detected incidence of viruses in the study population*

	Antibody responses*	Isolations	Antibody responses and/or isolations
Influenza A	55	15	59
Rhinoviruses	N.A.	3	3
Coronavirus OC-43	5	N.A.	5
Adenoviruses	17	2	18
Respiratory syncytial virus	24	3	27

* Fourfold or greater rise in antibody titre.

anxiety and slight increase of blood pressure – 1·5 MU group. Two withdrew for unrelated personal reasons. The data from all who withdrew were included in the analysis up to the time they left the study.

Analysis of reported symptoms showed significant differences between groups for the occurrence of blood-tinged mucus and nasal stuffiness (Kruskal–Wallis test statistics of 28·84 and 9·71, 3 degrees of freedom for each; $P < 0{\cdot}0001$ and 0·0212, respectively). Casual examination of cumulative rank sums showed the trend to be increasing with dose (Table 3). Analysis of the weekly numbers of individuals with blood-tinged mucus using the generalized linear model showed a significant effect in relation to weeks ($\chi^2_4 = 9{\cdot}50$; $P = 0{\cdot}0497$) and, more significantly, in relation to dose ($\chi^2_3 = 68{\cdot}085$; $P < 0{\cdot}000\,001$). The interactive effect of dose and week was not significant ($\chi^2_4 = 3{\cdot}429$). From Table 3, it can be seen that, in general, the presence of blood-tinged mucus increased with the passage of time and decreased in week 5 when treatment was discontinued. To determine the overall weekly average for analysis, a person having blood-tinged mucus on any number of days was included only once for each week in which the symptom occurred.

Analysis of the post-trial survey data indicated that nasal discomfort was significantly different between groups ($\chi^2_9 = 21{\cdot}097$; $P = 0{\cdot}012$); the proportions of individuals reporting some degree of discomfort (placebo, 37% 1·5 MU/day, 44%; 3·0 MU/day, 59%; 6·0 MU/day, 59%) suggest this was more commonly associated with 3·0 and 6·0 dosages.

The incidence of the most commonly detected viruses in the study population is shown in Table 4. Virus isolations were only attempted on samples from the 107 individuals who presented for acute visits.

An outbreak of influenza A/Victoria/3/85 (H3N2) occurred in both the study population and general population of Newcastle during the trial, which accounts

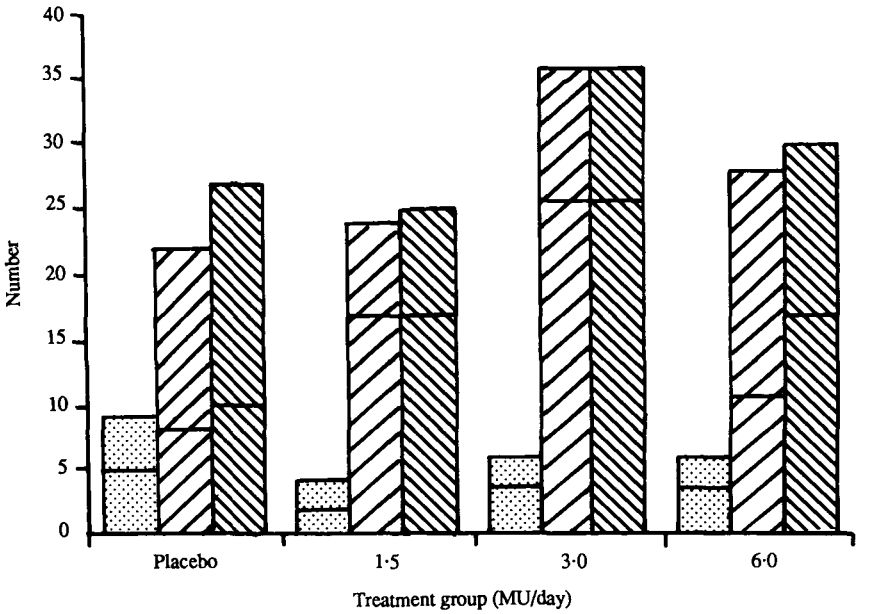

Fig. 1. Number with laboratory-documented illnesses by isolation and/or antibody response. Isolations are indicated by ▒, antibody responses by ▨, and either isolations or seroconversations by ▧. The lower portion of each column is the number for influenza A.

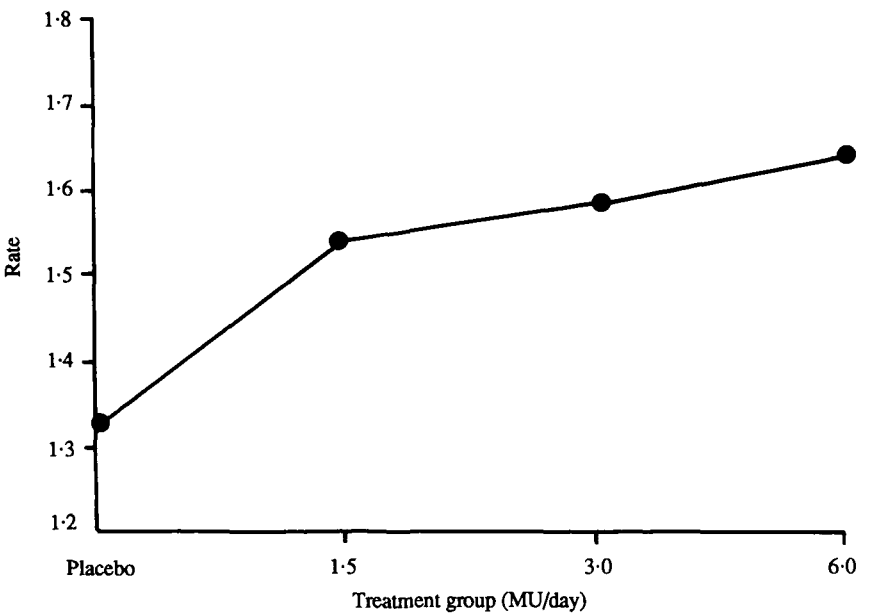

Fig. 2. Average number of antibody responses among antibody responders in treatment groups. Each point is obtained by dividing the total number of antibody responses per group by the number of individuals responding.

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Table 5. *Relative risk of trial outcome variables*

Factor	Relative risk*	95% Confidence interval
Isolations	0·585	0·267–1·283
Antibody responses	1·316	0·873–1·983
Isolation and/or antibody responses	1·109	0·769–1·600
URTE	1·097	0·798–1·508
GIE	0·947	0·683–1·313
Acute samples taken	0·845	0·591–1·208
Nasal erosions	1·585	0·862–2·917

* Based upon comparisons between the outcome data for the placebo group and the combined interferon groups.

for the higher number of isolations and antibody responses to that virus. However, the incidence of respiratory syncytial virus (the next most prevalent virus) is surprisingly high for an adult population.

The distribution of viral infections by treatment group is shown in (Fig. 1). There was a higher incidence of antibody responses and lower incidence of isolation in the treatment groups compared to the placebo group. However, these differences were not statistically significant. Some subjects had more than one laboratory confirmed infection during the course of the trial as shown by the total number of antibody responses in Fig. 1. Although there was no difference in the incidence of URTE and GIE between treatment and placebo groups (Table 2), the difference in antibody responses (Fig. 2) suggests a higher incidence of asymptomatic illness in all three treatment groups. Using analysis of variance for linear regression on transformed seroconversion rates, a significant dose response relationship was found. ($F = 27{\cdot}6856$; $P = 0{\cdot}034$; coefficient of determination 93%) (Zarr, 1974; Coulton, 1974). The overall rate of virus isolation and/or antibody responses in the four medication groups (placebo, 1·5, 3·0 and 6·0 MU per day) was 57, 36, 54 and 52% respectively, which compares favourably with standard diagnostic experience (data not shown).

The summary data in Table 5 are based on comparisons between the outcome data of the placebo group and the combined interferon groups, expressed as relative risk with 95% confidence intervals. Gross comparison of these outcome measures revealed no statistically significant differences. However, the relatively large confidence intervals suggest that a larger sample size may have resulted in statistically significant findings, especially in relation to isolations, antibody responses and the occurrence of nasal erosions.

DISCUSSION

In this randomized controlled trial, no statistically significant benefit was shown for the use of intranasally administered interferon in the prophylaxis of respiratory tract infection using either symptom-based diagnoses of upper respiratory illness or laboratory-based outcome criteria. However, a significant relationship was found between the dose of interferon and the side-effects of nasal bleeding and nasal stuffiness. The trend in relation to detected nasal erosions was inconsistent, although erosions were more common in treatment groups.

The total discomfort experienced over the trial period was relatively small and there were only three drug-related withdrawals out of an initial population of 412. In addition, only two participants had erosions sufficient to require cauterization at the end of the study period. One of these had been receiving 1·5 MU daily and the other the placebo.

Outcome measures were used on both reported symptoms or for the detection of a virus. The difficult task of detecting true episodes of respiratory tract infection from symptoms produced by local side-effects of interferon and other symptoms was managed by using an algorithm. Any misinterpretation of episodes from the algorithm would have been evenly distributed between the groups, since a reporting bias is unlikely in a double-blind and placebo-controlled trial. Although known dose-related symptoms were excluded from the algorithm, it must be acknowledged that the validity of the clinical outcome alone is open to question when a drug produces side-effects that are very similar to the illness it is supposed to prevent. On the other hand, nasal symptoms are very important to a patient with an upper respiratory tract infection.

Isolation procedures were carried out only on acute specimens, which meant considerable reliance on subject cooperation. In practice, acute nasal and throat washings were not always taken. However, acute samples for viral studies were obtained from a smaller proportion of symptomatic subjects in the treatment groups compared to the placebo group. Since this trend would tend to exaggerate the efficacy of interferon, it does not affect the conclusions of the study.

The virological data indicated that an influenza A outbreak had commenced just prior to the start of the trial. It is possible that illness with this virus and also with adenovirus and respiratory syncytial virus may have reduced the instances of infection by rhinoviruses or coronaviruses, which are considered to be responsible for most common-cold-like illnesses (Phillpotts & Tyrrell, 1985). Interferon appeared to provide no benefit in the amelioration of respiratory illnesses caused by those viruses present during this trial. Monto *et al.* (1986) also described a 4-week study in which intranasally administered interferon had no prophylactic efficacy against parainfluenza, respiratory syncytial and coronavirus infections. Two family studies (Douglas *et al.* 1986; Hayden *et al.* 1986) also showed that intra-nasal interferon had no protective effect against influenza viruses A or B, parainfluenza viruses or coronaviruses.

Symptoms which are considered to be side-effects of interferon and, in particular, nasal stuffiness and blood-tinged mucus, were found to be related to dose. The absence of a dose–response relationship for nasal erosions may be explained by the higher than expected occurrence of erosions in the placebo group. It is possible that this was due to benzalkonium in the placebo and that an inert placebo, such as normal saline, would not have produced as much nasal irritation. Alternatively, erosions and bleeding could have been caused by repeated use of the spray and associated mechanical trauma.

Although there was no statistically significant difference between the placebo and the combined interferon groups, there were interesting trends in the results with respect to viral isolation and antibody response. Viral isolation was less in the interferon group than the placebo (relative risk (RR) 0·585) but antibody responses occurred more often (RR 1·316), both in the number of subjects and the

multiplicity of illnesses. One hypothesis arising from these results is that interferon does not stop the initial infection as detected by an antibody rise, but reduces viral multiplication detected by viral isolation from nasal washings. Enhancement of the antibody response which was observed with increasing doses could indicate some role for interferon as an adjuvant or it may occur as a consequence of damages to the nasal mucosa following administration.

In view of the lower than anticipated rate of viral isolation by culture, it is possible that a false negative result or Type II error occurred due to a smaller than expected sample size (relative risk and confidence intervals: 0·585; 0·267–1·283). A larger sample size could have detected a significant effect of interferon in reducing viral upper respiratory infection. The results of two risk groups support this possibility (Douglas *et al.* 1986; Hayden *et al.* 1986). In these studies medication was only administered for 7 days which may have resulted in a reduction of side-effects. However, other studies in which interferon A was administered for several weeks suggest that, despite decreases in the incidence of rhinovirus infections, there was no net benefit because of adverse effects from the medication (Farr *et al.* 1984; Douglas *et al.* 1985; Hayden, Gwaltney & Johns, 1985).

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