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Tolerance and nasal histopathologic effects of long-term, low-dose intranasal recombinant interferon alpha-2A (Roferon-A)

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Summary

The dose-related tolerance and histopathologic effects of intranasal recombinant interferon alpha-2a (rIFN-α2a) were determined in a blind study in which healthy subjects were randomized to receive sprays of interferon (IFN) (3 or 6 MU/day) or placebo for 28 days. Adverse nasal symptoms (bleeding, obstruction, irritation) tended to occur more often in the IFN 6 MU/day group. Blind analysis of biopsy samples collected before and after IFN treatment revealed that both IFN groups had significant histologic changes, most commonly an increased degree of lymphocytic infiltration in the subepithelium and underlying glandular zones. Changes developed in 61% of 18 recipients of IFN 6 MU/day, 37% of 19 recipients of IFN 3 MU/day, and 6% of 18 placebo recipients. Serum antibodies to rIFN-α2a detectable by EIA were found in 4 recipients of IFN 3 MU/day and 2 recipients of IFN 6 MU/day, one-third of whom were positive by neutralization bioassay. The findings would predict that these rIFN-α2a dosages would be associated with an excess of adverse side effects during long-term use in healthy adults.

Intranasal recombinant interferon alpha-2a; Tolerance; Histopathologic effects

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Introduction

Intranasal recombinant interferon alpha-2b (rIFN-α2b) has been shown to be effective for prevention of rhinovirus infections when used on a daily basis for long-term prophylaxis (Betts et al. 1983; Farr et al., 1984; Douglas et al., 1985; Monto et al., 1986) or for postexposure prophylaxis in the family setting (Douglas et al., 1986; Hayden et al., 1986). However, administration is limited by dose- and duration-related nasal intolerance. Local toxicity is manifested by complaints of nasal stuffiness, blood-tinged mucus, dryness and discomfort, and by mucosal signs of friability, hemorrhage, erosion and ulceration. Clinical intolerance has been observed within 2 wk of initiating exposure to intranasal recombinant leukocyte A interferon (rIFN-α2a) (Samo et al., 1983; 1984), rIFN-α2b (Betts et al., 1983; Hayden et al., 1983, 1985, 1986; Farr et al., 1984; Douglas et al., 1985, 1986; Monto et al., 1986), and human leukocyte-derived interferon (Scott et al., 1985).

One study assessing nasal mucosal histopathology before and after interferon exposure revealed that the majority of recipients of rIFN-α2b 8 MU/day developed moderate or marked chronic inflammatory change, principally lymphocytic infiltration, in the subepithelium of biopsy specimens after 28 days of exposure (Hayden et al., 1983). A smaller portion developed acute inflammatory change in the epithelium or epithelial ulceration. In another study it was found that increased degrees of lymphocytic infiltration occurred as early as 4 days, and in the majority of recipients by 10 days, after initiating exposure to rIFN-α2a 9 MU/day (Hayden et al., 1987). In these studies many subjects with histopathologic abnormalities did not have associated symptoms of intolerance (Hayden et al., 1983, 1987).

The histopathologic effects of lower interferon dosages have not been assessed previously. The current studies determined the dose-related tolerance and histopathologic effects of rIFN-α2a when given on a daily basis for a period of 4 wk.

Methods

Participants

Healthy adult volunteers were recruited for participation. Those with recent upper respiratory tract infection or active allergic symptoms were excluded. Intranasal medications of any type and oral antipyretics and anti-inflammatory agents were excluded for 2 wk before treatment and during the study. Nasal examinations were performed before and at weekly intervals during the treatment period. Blood specimens for routine hematology and chemistry studies (gamma glutamyl transpeptidase, alanine aminotransferase), and sera for assay of antibody to rIFN-α2a were collected before and on the last treatment day. Measurements for antibody were done by both enzyme immunoassay (EIA) and by bioassay (Hennes et al., in press). An interferon concentration of 10 IU/ml was used for the bioassay, and the detection limit was 100 neutralizing units (NU)/ml.

Interferon administration

Recombinant leukocyte A interferon (rIFN-α2a) was reconstituted in 6 ml volumes at concentrations of 4.0 or 8.0 × 10^6 IU/ml. The excipients, which included sodium chloride, human serum albumin USP, and the preservative benzalkonium chloride 0.1%, served as the placebo solution. Under double-blind conditions, the subjects were randomly assigned to receive intranasal sprays of rIFN-α2a 6 MU/day or 3 MU/day or placebo. A metered pump spray was used to deliver the study drugs in 2 sprays (0.09 ml/spray) per nostril per treatment. Sprays were self-administered by the subjects twice daily for 28 consecutive days. The sprayers were refrigerated between uses.

Nasal biopsies and histopathologic analysis

Punch nasal biopsies (≈ 3 mm in diameter) were obtained from the anterior portion of the inferior turbinate of each participant before and after interferon exposure. Specimens were fixed in formalin, embedded in paraffin, and sections stained with hematoxylin and eosin.

The histologic features of the biopsies were assessed independently by two pathologists (D.J.I. and S.E.M.), who were blinded as to the timing of the biopsy and treatment status of the subjects. Using modifications of previously described techniques (Hayden et al., 1983, 1987), the pathologists assessed the different levels of the biopsy specimens (epithelium, subepithelium, gradular zone, and deep vascular zone) for the degree and type of inflammatory cell infiltrate. The degree of infiltrate was ranked on a 4-point scale, (0–3, absent-marked). The predominant cell types (polymorphonuclear leukocytes, lymphocytes, or plasma cells) and the presence of edema, hemorrhage, or vascular changes in the deeper portions of the biopsy were noted. The type of epithelium (ciliated, squamous, mixed or intermediate) and the presence of atrophy or epithelial ulceration were also noted. For purposes of analysis, the postexposure biopsy findings were compared to those of the preexposure biopsy on a subject-by-subject basis. Biopsy pairs which showed at least a one grade increase in score (e.g., 0–1, 1–2, or 2–3) according to both pathologists were considered to show histologic change.

Statistical analysis

The Wilcoxon 2-sample test with a continuity correction of 0.5 was used for comparisons of histologic scores between the two treatment groups. The signed ranks test was used to compare scores before and after exposure within each treatment group. Fisher's exact test was used to compare proportions between the groups. In each instance *P*-values are those for 2-tailed testing.

Results

Participants

Fifty-six healthy adult volunteers were recruited for participation at two study sites (26 in Basel, 30 in Dublin). These groups were comparable in demographic

TABLE 1

Demographic characteristics of participants.

	Treatment group		
	Placebo	IFN 3 MU/d	IFN 6 MU/d
No. subjects	19	19	18
No. females	12	10	9
Age (years)[a]	26±6	26±8	29±8
Weight (kg)[a]	61±9	63±14	68±13
Height (cm)[a]	170±8	168±8	171±9
No. smokers	5	7	8
No. allergy history	1	0	1
No. drop-outs	0	0	1[b]

[a] Mean ± S.D.

[b] Removed from study after 3 wk with complaints of nasal irritative symptoms and facial swelling.

characteristics (Table 1). The distribution of subjects from the 2 study centers were similar in each of the placebo (9 Basel, 10 Dublin), IFN 3 MU/day (9 Basel, 10 Dublin), and IFN 6 MU/day (8 Basel, 10 Dublin) groups.

All subjects completed the 28-day dosing period, except for one subject in the IFN 6 MU/day group who was removed after 20 days because of severe complaints of nasal irritation and facial swelling and nasal exam findings of inflamed mucosa with hemorrhage and ulceration. This individual's nasal biopsy results were included in the analysis.

Clinical and laboratory abnormalities

Relatively few subjects reported adverse nasal symptoms during the course of drug administration (Table 2). IFN 6 MU/day recipients tended to have higher frequencies of complaints of bleeding (bloody nose, bloodtinged mucus, or spotting), nasal obstruction (stuffiness or blocked nose), or irritative symptoms (dry, sore,

TABLE 2

Clinical and laboratory abnormalities during intranasal rIFN-α2a administration.

	No. of subjects		
Parameter	Placebo	IFN 3 MU/day	IFN 6 MU/day
Adverse nasal symptoms			
Bleeding[a]	2	2	4
Obstruction	3	3	5
Irritation[b]	1	1	4
Nasal exam signs (4 wk)[c]	1	1	3
Serum antibody to rIFN-α2a			
EIA	0	4	2
Bioassay	0	2	0

[a] Symptoms = bloody nose, blood-tinged mucus, or spotting.

[b] Symptoms = dry, sore, or itchy nose.

[c] Punctate bleeding sites, erosions, ulcerations.

TABLE 3

Nasal biopsy changes after intranasal rIFN-α2a for 4 wk.

Treatment group	No. showing inflammatory change/total no. evaluable biopsies (%)[a]				
	Epithelium	Subepithelium	Glandular zone	Deep vascular zone	Any level
Placebo	1[b]/15 (7)	1/18 (6)	0/17 (0)	0/13 (0)	1/18 (6)
IFN 3 MU/day	2/18 (11)	7/19 (37)[c]	2/19 (11)	0/11 (0)	7/19 (37)[c]
IFN 6 MU/day	4[b]/16 (25)	10/18 (56)[d]	5/17 (29)[c]	0/13 (0)	11/18 (61)[d]

[a] Increase in degree of inflammation compared to pre-exposure biopsy.
[b] 1 with ulceration.
[c] $P<0.05$, versus placebo.
[d] $P<0.01$, versus placebo.

or itching nose), compared to the other groups. Nasal exams conducted at the end of the dosing period found surprisingly few abnormalities, particularly in the group enrolled in Dublin, in whom no postexposure abnormalities were found.

Clinically significant laboratory abnormalities were not recognized. One IFN 6 MU/day recipient developed a slight decrease in platelet count (145,000/mm^3). In contrast to studies conducted with higher dosage of rIFN-α2b (Farr et al., 1984),

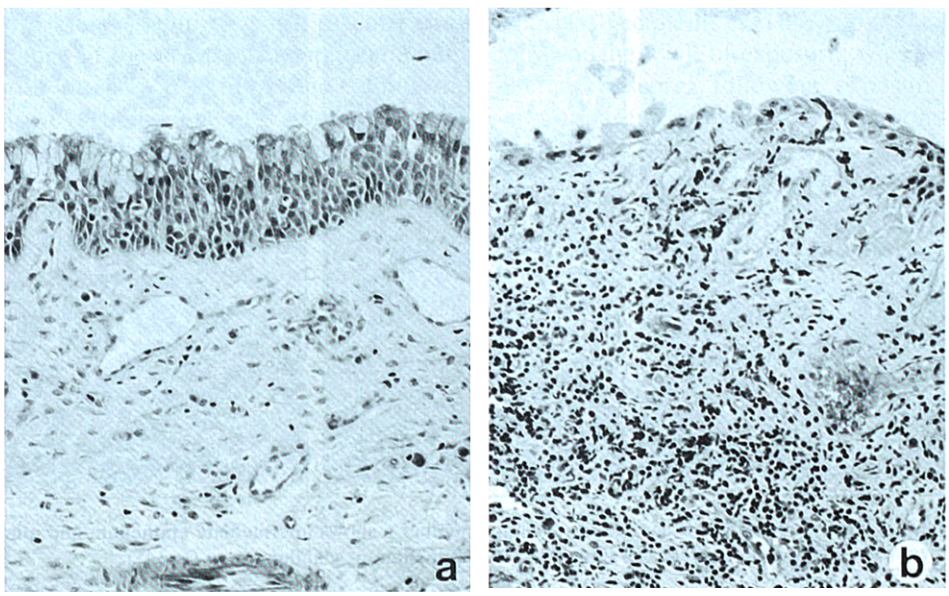

Fig. 1a. Pre-exposure biopsy from subject numer 1 shows normal respiratory epithelium and scant mononuclear cell infiltrate in the subepithelial and glandular zone (× 145).

Fig. 1b. Biopsy from subject number 1 after rIFN-α2a 3 MU/day treatment for 4 wk shows moderate to marked mononuclear cell infiltrate in subepithelium and glandular zone (× 145). Epithelium is partially stripped from biopsy sample.

no instances of leukopenia or significant changes in total white blood cell or platelet counts were recognized.

Three interferon-treated subjects at each study site (4 receiving IFN 3 MU/day, 2 receiving IFN 6 MU/day) developed serum antibodies to rIFN-α2a by EIA. These antibodies were not present in preexposure samples and were detected in samples collected on both the last day of dosing and 1 wk later. Two of the 6 individuals also had low levels of neutralizing antibody (200 and 600 NU/ml) demonstrated by bioassay. No clinical findings were associated with the development of the antibodies.

Histologic findings

As shown in Table 3, both interferon groups had significant histologic changes in nasal biopsy specimens taken after 4 wk of exposure, compared to those taken before exposure or to placebo recipients. Although not all portions of each biopsy were evaluable because of trauma or varying biopsy size, changes were found most frequently in the subepithelial area and least often in the deep vascular stroma.

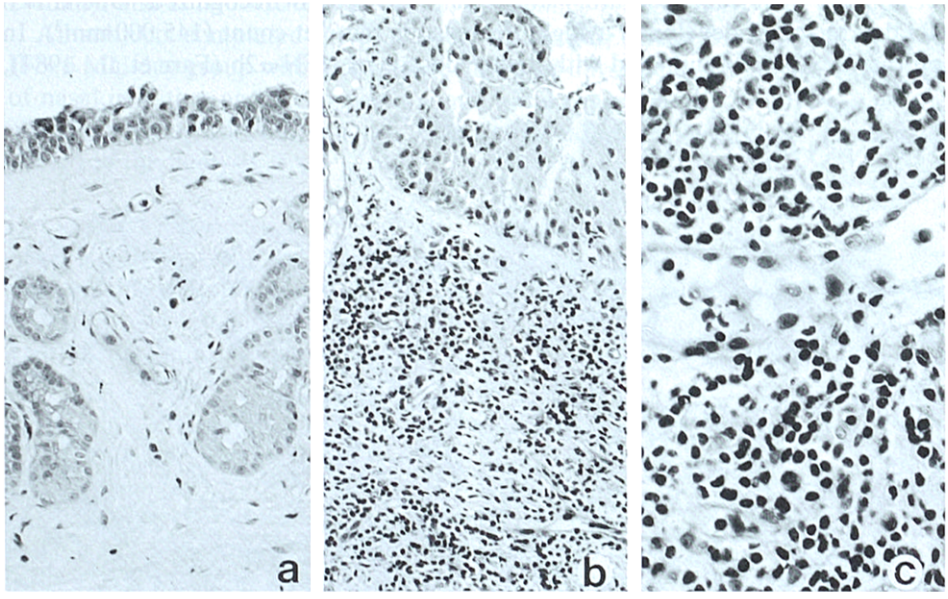

Fig. 2a. Pre-exposure biopsy sample from subject number 6 shows intermediate epithelium and minimal mononuclear cell infiltrate in subepithelium or glandular area (× 145).

Fig. 2b. Post-exposure biopsy sample from subject number 6 following treatment with rIFN-α2a 6 MU/day shows dense mononuclear cell infiltration in subepithelium and glandular zone and scattered polymorphonuclear neutrophils in the epithelium (× 145).

Fig. 2c. High-powered view of the subepithelial area from the post-exposure biopsy of subject number 6 (× 290) shows marked infiltration by small lymphocytes. Blood vessel in center of section shows reactive changes with enlarged endothelial cells.

TABLE 4

Degree of lymphocytic infiltration in nasal biopsy specimens after intranasal rIFN-α2a.

	Mean score change[a] in			
	Subepithelium		Glandular zone	
Treatment group(n)	Pathologist 1	Pathologist 2	Pathologist 1	Pathologist 2
Placebo (18)	0.1	−0.1	0.1	−0.1
IFN3 (19)	1.2[b]	1.1[b]	0.6[c]	0.8[b]
IFN6 (18)	1.2[b]	1.3[b]	0.9[b]	0.6[c]

[a] Mean of post-exposure score - pre-exposure score for each pair of biopsies.
[b] $P<0.01$, interferon group versus placebo and $P<0.01$ pre-exposure versus post-exposure.
[c] $P<0.05$, interferon group versus placebo and $P<0.01$ pre-exposure versus post-exposure.

The most common pattern was an increased degree of lymphocytic infiltration in the subepithelium and underlying grandular zone (Figs. 1 and 2). Overall, both pathologists independently identified increased degrees of chronic inflammation, compared to the corresponding preexposure biopsy, in 6% of 18 placebo recipients, 37% of 19 IFN 3 MU/day ($P<0.05$ vs placebo), and 61% of 18 IFN 6 MU/day recipients ($P<0.01$ vs placebo). As shown in Table 4, each pathologist found significant increases in the degree of lymphocytic infiltration in the subepithelium and glandular zone of interferon recipients compared to the corresponding preexposure biopsies and also the placebo group. Placebo recipients had no substantial change in the degree of lymphocytic infiltration over the 4 wk of exposure, whereas both interferon groups had significantly increased scores following exposure. However, evidence of a dose-response was not apparent, when scores were compared between interferon groups.

Except for certain vascular endothelial changes in interferon recipients (Fig. 2c), no differences in the occurrence of other histologic abnormalities were recognized between the groups (Table 5). Reactive vascular endothelium was seen in 10 of 37 (27%) postexposure biopsy samples from interferon recipients. All of these biopsies also showed a moderate (score=2) or marked (score=3) degree of lymphocytic infiltration. In one instance a preexposure biopsy showed this change in association with a moderate lymphocytic infiltrate.

TABLE 5

Other histologic findings in nasal biopsy specimens before and after intranasal rIFN-α2a.

	No. of biopsies					
	Placebo		IFN 3 MU/day		IFN 6 MU/day	
Finding (Number of biopsies)	Pre (19)	Post (18)	Pre (19)	Post (19)	Pre (18)	Post (18)
Hemorrhage	2	1	0	1	0	1
Edema	3	3	0	1	0	1
Fibrosis	1	2	1	0	0	1
Reactive vascular endothelium	0	0	1	5[a]	0	5[a]

[a] $P<0.05$, versus placebo.

Inflammatory changes in the superficial epithelium of the biopsies were seen less often than in the deeper areas (Table 3). In contrast to the lymphocytic mononuclear cell infiltration observed in the deeper portions of the biopsy, epithelial changes were associated with increased numbers of polymorphonuclear leukocytes (Fig. 2b). Atrophic changes were not recognized, and ulcers were seen infrequently. The development of inflammatory changes in the epithelium after spray exposure, including the one instance in a placebo recipient, was seen only in biopsies that also showed chronic lymphocytic infiltrations of mederate or marked degree in the subepithelium. On the other hand, a majority of biopsies showing subepithelial or glandular chronic inflammatory change did not have obvious epithelial changes.

Relationship of histologic changes to clinical findings

The occurence of nasal irritative symptoms was predictive of histologic changes in interferon recipients. Of the 6 interferon recipients who had symptoms of nasal bleeding or blood-tinged mucus, 5 (83%) had histologic changes on their post-exposure biopsy specimens, as compared to 14 of the 31 (45%) who did not report nasal bleeding ($P=0.18$). Of 8 subjects reporting nasal obstruction or stuffiness, 7 (88%) had histologic changes, compared to 12 of 29 (41%) who did not report such symptoms ($P=0.04$). All 5 subjects (100%) who reported symptoms of nasal irritation had histologic changes, compared to 14 of the 32 (44%) who did not report such symptoms ($P=0.05$). Overall, 11 of the 13 (85%) interferon recipients who reported one or more adverse nasal symptoms had histologic changes on nasal biopsy specimens, compared to 8 of 24 (33%) who did not report symptoms ($P=0.005$).

In contrast, other variables did not correlate positively with the development of histologic changes in interferon recipients. The proportion of recipients who developed histologic changes were similar in males (9 of 18, 50%) and females (9 of 19, 47%) or in those who were biopsied in Dublin (9 of 20, 45%) and Basel (8 of 17, 47%). Of the 6 interferon recipients who had developed serum antibodies to rIFN-α2a (Table 2), only 2 had nasal biopsies which showed histologic changes. However, smoking status did affect the frequency of histologic findings. A lower proportion of smokers developed histologic abnormalities (4 of 16, 25%) than did non-smokers (14 of 22, 64%) ($P=0.04$).

Discussion

These studies utilized dosages of rIFN-α2a which had been shown for rIFN-α2b to be effective for prophylaxis of rhinovirus colds but also associated with symptoms of nasal adverse effects after several weeks administration (Douglas et al., 1985; Monto et al., 1986). Although statistically significant differences in clinical tolerances were not found in this study, in part because of the relatively small sample sizes, the findings indicated that long-term administration of rIFN-α2a at a dosage of IFN 6 MU/day was associated with more symptoms and signs of local

intolerance than either placebo or the 3 MU/day dosage. Earlier efficacy studies of rIFN-α2b at a dosage of 10 MU/day had to be terminated after 3 wk because of severe local side effects (Betts et al., 1983; Farr et al., 1984), and studies utilizing dosages of 2–3 MU/day also found signs and symptoms of nasal adverse effects in 40% or more of recipients at 2–4 wk (Douglas et al., 1985; Monto et al., 1986). Following exposure to intranasal rIFN-α2a at a dosage of 2.4 or 10 MU/day, 15 and 26% of healthy volunteers, respectively, developed evidence of nasal intolerance within 2–4 wk (Samo et al., 1983, 1984).

Confirming observations of earlier studies (Hayden et al., 1983, 1987), significant histopathologic alterations were observed in the postexposure biopsies of both interferon groups. The placebo recipients, who were treated on a daily basis with the excipient solution, manifested no significant histopathologic changes over the course of the study. In contrast, both interferon groups had significant numbers of subjects who showed increases in lymphocytic infiltration of a moderate or marked degree. Although the proportion of subjects who had such increases in their biopsy samples tended to be higher in the 6 MU/day group compared to the 3 MU/day group, no dose-response was seen when the average changes in score were compared (Table 4). The character of the histologic changes, principally consisting of increased numbers of lymphocytic cells in the subepithelium, and to a lesser extent in the glandular zones, in the biopsies was consistent with the observations made in individuals receiving intranasal rIFN-α2b (Hayden et al., 1983). The findings of the current study would predict that rIFN-α2a in these dosages would be associated with an excess of adverse effects during long-term use.

As shown in earlier studies (Hayden et al., 1983, 1987), the majority of interferon recipients who had histopathologic changes with increased degrees of lyphocytic infiltration had neither epithelial changes nor symptoms of local intolerance. However, almost all symptomatic individuals had histopathologic changes. These observations indicate that the histologic changes precede the clinical signs of intolerance. Epithelial changes were seen less often than changes in the deeper portions of the biopsies (Table 3) and were primarily related to acute inflammatory changes, infrequently associated with ulceration. This typographic analysis indicates that the subepithelial changes are not simply due to epithelial irritation but likely lead to changes in the overlying epithelium.

The mechanisms by which interferon induces histologic changes and subsequent clinical intolerance are uncertain. Interferons have a wide range of immunomodulating effects, including activation of macrophages, increased cell-mediated cytotocity and natural killer cell activity, and alterations of membrane antigen expression (DeMaeyer et al., 1984; Shalaby et al., 1984). Parenteral administration of interferon in mice causes reduction in thoracic duct lymphocyte counts and increases in lymph node size and cell content (Korngold et al., 1983; Gresser et al., 1981). Such changes are hypothesized to be secondary to inhibition of lymphocyte egress from lymph nodes. In a related observation in oncology patients, parenteral treatment with rIFN-α2 is associated with significant increases in lymphocyte content of bone marrow samples (Ernstoff and Kirkwood, 1984). Intranasal interferon may possibly affect the movement of lymphocytes through the na-

sal mucosa, perhaps by augmenting their migration and/or preventing their egress. The fraction of patients who develop histologic changes may have a higher degree of responsiveness to topical interferon, or interferon may be augmenting a response to another local stimulus.

The observation that histologic changes developed less often in interferon recipients who smoked, than in those who did not, is of interest. Although an obvious explanation for this apparent difference is lacking, it may be that smoking provides some protection against local intolerance by affecting the distribution, clearance or biologic activity of intranasal interferon. In this regard, it would be important to know if smokers treated with intranasal interferon develop comparable antiviral protection compared to nonsmokers.

Previous studies utilizing a commercially available immunoradiometric assay (IRMA) with modifications (Protzman et al., 1984) found evidence of transient circulating antibody in less than 1% of over 1300 intranasal rIFN-α2b recipients (Spiegel et al., 1986). In contrast, the current study found that 6 of 37 (16%) interferon recipients developed antibody to EIA and 2 of 37 (5%) by bioassay. These findings suggest either that rIFN-α2a is more immunogenic than rIFN-α2b, despite the similarity of the molecules, or that the direct assay methods used in the current study are more sensitive than the competitive binding assay used in the studies of rIFN-α2b (Spiegel et al., 1986). A recent comparative study of different assay methods for measuring serum antibodies to interferon found that the IRMA was much less sensitive than the EIA used in the current study (Itri et al., 1987) and that the IRMA failed to detect antibody in nearly one-half of sera positive by EIA and conventional neutralization bioassay. In the current study, the development of antibody did not appear to correlate with the histopathologic effects seen in the biopsy samples, nor was it associated with clinical intolerance. The clinical significance of this immunologic response to intranasal interferon is uncertain but warrants careful study.

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