Parenteral Inactivated Rhinovirus Vaccine: Minimal Protective Effect[1] (36262)

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Rhinoviruses are the most frequently isolated agents from individuals with common colds (1). Prevention of such infections by immunization presents a formidable problem because of the multiplicity of serotypes, and the lack of epidemiologically predominant serotypes (1).

Since no other approaches to control are currently available, and since prior studies have given conflicting results, (2–6) we systematically examined the response of volunteers to an inactivated parenterally administered rhinovirus type 13 vaccine. It was shown that this vaccine is highly antigenic in terms of serum antibody, but only weakly antigenic in terms of nasal secretory antibody production. In a controlled challenge study using a small inoculum dose, slight reduction in virus shedding and in severity and duration of illness was observed, but frequencies of illness and of infection were not significantly reduced.

Materials and Methods. Studies were performed at the Ramsey Unit of the Texas Department of Corrections. Procedures for acquiring volunteers and performing studies in the institution are essentially the same as those previously described (7, 8).

Vaccines. Rhinovirus type 13 vaccine, prepared by Abbott Laboratories on 3/11/64, lot no. 797-8930, had an infectivity titer of $10^{5.0}$ fifty percent tissue culture infectious doses ($TCID_{50}$) per milliliter (ml) prior to formalin inactivation. We administered this vaccine subcutaneously in three 1.0 ml doses at two week intervals. Volunteers were alerted to report to sick call if any significant reaction occurred, and were questioned at each vaccination regarding local or systemic side effects from previous vaccinations.

Clinical evaluation. Volunteers who were inoculated with live virus were examined daily by physicians who knew only the date of inoculation. Oral temperatures were recorded every 12 hr. A designation of respiratory illness was based on the daily evaluations, and graded according to severity (1+, 2+ or 3+) predominant site of involvement (rhinitis, pharyngitis, tracheobronchitis or systemic illness), and occurrence of fever ($\geq$ 100°F).

Challenge pool. The challenge pool of type 13 was prepared from second passage harvests of human embryonic fibroblast cultures (WI-38), and safety-tested as previously described (9). Inoculation of volunteers was by nasal drops, 0.25 ml per nostril. The 50% human infectious dose (HID_{50}) of this inoculum is 0.10 $TCID_{50}$ (10).

Virus isolations and identifications. Nasal wash specimens were collected and tested in WI-38 cultures as previously described (9). The first and last virus isolate from each volunteer was identified by neutralization tests (9).

Neutralization tests and immunoglobulins. Nasal secretions (NS) were processed as previously described (11), and concentrated approximately tenfold by ultrafiltration in dialysis tubing against dry Sephadex G-200 powder. This method was shown to provide concentration without loss of IgA or neutralizing activity. The mean IgA level in concentrated specimens was 0.340 mg/ml, and the

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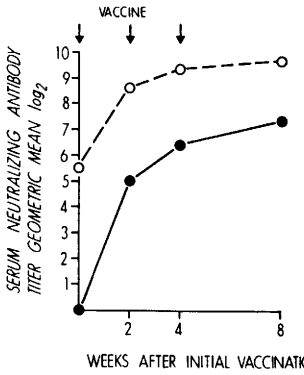

FIG. 1. Serum neutralizing antibody responses to rhinovirus type 13 vaccine. Twenty-nine volunteers (●—●) had low (1:2) or undetectable levels at start of vaccination; 17 others (○ - - - ○) had titers ranging from 1:4 – 1:512 (mean 1:44).

range 0.125–1.78 mg/ml.

Sucrose density gradient ultracentrifugation was performed in a model L-2 ultracentrifuge at 60,000 rpm and 6–13°. Quantities of immunoglobulin A (IgA) were determined in each concentrated nasal secretion specimen, and in sucrose density gradient fractions by the single radial diffusion method against an 11S IgA standard.

Tests for neutralizing antibody were performed in WI-38 cultures by previously described methods (12). The lowest dilution of serum and concentrated NS tested was 1:2 and 1:4, respectively.

Results. Serum antibody response. Serum neutralizing antibody responses of 46 volunteers vaccinated with rhinovirus type 13 subcutaneously are shown in Fig. 1. Twenty-nine volunteers had low (1:2) or undetectable levels of serum antibody to rhinovirus type 13 at the time of vaccination, whereas 17 others had titers ranging from 1:4 to 1:512 (mean 1:44). As indicated, moderate to high mean levels of serum antibody developed rapidly in each group, and the responses of the two groups were parallel.

All but two volunteers with low or undetectable serum antibody titers prior to vaccination developed fourfold or greater rises in antibody titers two weeks after the first inoculation, and these two had developed antibody responses by the fourth week. One volunteer who possessed antibody initially did not develop a response until the eighth week. All others in the group with antibody prior to vaccination responded in two weeks.

Nasal secretion antibody responses. Two of 29 volunteers with low or undetectable serum antibody had detectable nasal secretory antibody (1:4) prior to vaccination. As indicated in Table I, 11 of these 29 volunteers developed rises in nasal secretory neutralizing antibody at two, four or eight weeks after vaccination, however, mean titers for the group at each interval was <4. Antibody was not consistently detectable in the same individual but was more frequently present at four weeks than at two or eight weeks.

Nasal secretory neutralizing antibody was detected prior to vaccination in specimens from 4 of 17 volunteers who possessed serum antibody prior to vaccination. As shown in Table I, nasal secretory neutralizing antibody rises were more frequently detected (59% vs. 38%) than in the low serum antibody group, but this difference was not significant ($p > .10$, 2×2 contingency table), and titers were similar in both groups. As in the serum

TABLE I. Homotypic Nasal Secretion Neutralizing Antibody Response to Rhinovirus Type 13 Vaccine.

Serum antibody titer at initial vaccination	No. of volunteers	Nasal secretion antibody[a]						
		No. of volunteers with antibody rise[b]	Highest titer detected					
			4	8	16	32	64	128
<2–2	29	11 (38%)	3	3	2	1	2	
4–512	17	10 (59%)	5	2	1		1	1

[a] Specimens collected at 2, 4, and 8 weeks tested. All specimens concentrated tenfold.
[b] ≧ 4-fold.

TABLE II. Response of Volunteers to Nasal Challenge with Rhinovirus Type 13 Inoculum = 3 $TCID_{50}$.

Group	No. of volunteers	Virus shedding		Illness		
		No. of volunteers	Mean no. of isolates per volunteer	No. of volunteers	Mean duration (days)	Mean severity
Controls	13	12	5.1	9	4.3	1.5
Type 13 vaccine parenteral inactivated	14	9	2.9	6	2.8	0.7
		$p > .10$[a]	$p < .05$[b]	$p > .10$[a]	$p < .05$[b]	$p < .05$[b]

[a] Fisher exact test.

[b] Yates mean score test.

antibody-free group, the frequency of occurrence of nasal antibody was highest at four weeks.

Sucrose density gradient ultracentrifugation studies were performed on four of the concentrated nasal secretion specimens with the highest neutralizing titers obtained from volunteers who were originally antibody-free. In three specimens, neutralizing antibody predominantly sedimented in the 7S region; in the fourth specimen, 11S sedimenting neutralizing antibody which was associated with the presence of IgA predominated. In three, small amounts of heavier sedimenting activity, 15–19S, also were detected.

Challenge of volunteers with live rhinovirus type 13. Thirteen controls, and fourteen volunteers (originally antibody-free) who had been vaccinated with type 13 vaccine, beginning seven weeks earlier, were inoculated with live rhinovirus type 13, and the results are shown in Table II. The inoculum dose was 3 $TCID_{50}$ or 30 HID_{50}. At the time of inoculation, none of the controls possessed detectable nasal secretory or serum antibody to type 13. In the vaccinated group, serum neutralizing antibody titers ranged from 1:16–1:1024 and the mean was 1:128. Nasal secretory antibody (1:4, 1:8, 1:16, and 1:32) was detected in four of these volunteers.

As shown in Table II, the somewhat lower rates of infection and illness observed for the vaccinated group were not significantly different from controls. However, the mean number of isolates per volunteer, mean severity of illness and mean duration of illness were significantly reduced among volunteers who had received type 13 inactivated vaccines. Symptoms were similar in both groups.

In the vaccinated group, infection correlated inversely with magnitude of serum antibody (Yates mean score test, $x^2 = 4.27$, $p = .04$), but not to presence of nasal secretion antibody ($p > .50$). However, it was not possible to match out the effects of nasal neutralizing antibody because of the lack of volunteers who had low serum antibody and high nasal secretion antibody prior to challenge.

All twelve of the controls who shed virus developed fourfold serum antibody responses, and six developed nasal secretory antibody responses. In the vaccine group, four of nine volunteers who shed virus developed fourfold serum antibody responses. Four developed nasal secretion antibody responses, two of whom had serum antibody responses.

Discussion. In the present study we observed that rhinovirus type 13 administered subcutaneously in three doses two weeks apart resulted in high levels of serum antibody in 100% of vaccinated volunteers, but a low frequency and magnitude of antibody in nasal secretions. In addition, it was shown that the nasal antibody was predominantly 7S IgG rather than 11S IgA. Challenge of vaccinated volunteers with live rhinovirus type 13 resulted in no significant reduction in frequencies of infection and illness, but a significant reduction of virus shedding and duration and severity of illness was noted.

Previous studies have shown that volunteers given inactivated rhinovirus vaccine (type 1A or 2) parenterally developed homotypic serum antibody responses (2–6). In addition, some studies have shown protection against homotypic infection (3, 4, 6). However, in the study by Perkins *et al.* (2) illness and infection rates of volunteers vaccinated parenterally with rhinovirus type 13 were not significantly different from controls. A large challenge dose of virus was used, and it was suggested that failure to demonstrate protective effect after parenteral vaccination might be the result of the large inoculum. In the present study, the challenge dose of virus was in the order of magnitude that might simulate natural transmission and again no differences in rates of infection or illness were observed. However, by studying the severity and duration of illness and duration of virus shedding we were able to demonstrate vaccine effect.

It was shown that infection among vaccinees correlated inversely with magnitude of serum antibody and not with presence of nasal secretion antibody; thus, it seems likely that the limited protective effect observed may be attributed to serum antibody. Although it is possible that protection might have been greater among vaccinated volunteers with preexisting antibody because of the somewhat higher levels of antibody following vaccination, it seems more likely that protection in these individuals would also be limited since serum antibody levels were high in the antibody-free group after multiple immunizations, and neither the frequency nor height of nasal antibody titers was greater in the group possessing antibody prior to vaccination.

The finding of very limited protective effect of high levels of serum antibody after multiple immunizations together with the multiplicity of serotypes (105) of rhinovirus and the lack of prevalent serotypes make conventional parenteral vaccinations appear unpromising for control of rhinovirus common colds. Other approaches such as inactivated nasal vaccines, attenuated live vaccines, interferon inducers, and antiviral chemotherapeutic substances may offer more promise.

Summary. An inactivated preparation of rhinovirus type 13 administered subcutaneously to 29 antibody-free volunteers in three 1.0-ml doses at two week intervals resulted in rapid appearance of high titers of homotypic serum neutralizing antibody in 100% of volunteers. Parallel responses were observed in 17 other volunteeers with preexisting antibody. Antibody in nasal secretions was detected in 38% and 59% of vaccinees, respectively. Challenge of 14 vaccinees with 3 $TCID_{50}$ of live rhinovirus type 13 resulted in similar rates of infection and illness, as compared to 13 nonvaccinated controls, but there was significant reduction in virus shedding, severity of illness and duration of illness in the vaccinees.

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1. Hamre, D., *in* "Rhinoviruses, Monographs in Virology" (J. L. Melnick, ed.), Vol. 1. Basel, S. Karger AG (1968).

2. Parkins, J. C., Tucker, D. N., Knopf, H. L. S., Wenzel, R. P., Kapikian, A. Z., and Chanock, R. M., Amer. J. Epidemiol. **90**, 519 (1969).

3. Mufson, M. A., Ludwig, W. M., James, H. D., Jr., Gauld, L. W., Rourke, J. A., Holper, J. C., and Chanock, R. M., Amer. Med. Ass. **186**, 578 (1963).

4. Price, W. H., Proc. Nat. Acad. Sci. U.S.A. **43**, 790 (1957).

5. Doggett, J. E., Bynoe, M. L., and Tyrrell, D. A. J., Brit. Med. J. **1**, 34 (1963).

6. Andrewes, C. H., Brit. Med. J. **1**, 1344 (1965).

7. Fleet, W. F., Couch, R. B., Cate, T. R., and Knight, V., Amer. J. Epidemiol. **82**, 185 (1965).

8. Couch, R. B., Cate, T. R., Douglas, R. G., Jr., Gerone, P. J., and Knight, V., Bacteriol. Rev. **30**, 517 (1966).

9. Cate, T. R., Couch, R. B., and Johnson, K. M., J. Clin. Invest. **43**, 56 (1964).

10. Douglas, R. G., Jr., Ann. Otol. Rhinol. Laryngol. **79**, 563 (1970).

11. Cate, T. R., Rossen, R. D., Douglas, R. G. Jr., Butler, W. T., and Couch, R. B., Amer. J. Epidemiol. **84**, 352 (1966).

12. Douglas, R. G., Jr., Fleet, W. F., Cate, T. R., and Couch, R. B., Proc. Soc. Exp. Biol. Med. **127**, 497 (1968).

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