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Further Investigations of Live Respiratory Syncytial Virus Vaccine Administered Parenterally (40433)

EUGENE B. BUYNAK,* ROBERT E. WEIBEL,** ALFRED J. CARLSON,*** ARLENE A. MCLEAN,* AND MAURICE R. HILLEMAN*

*Division of Virus and Cell Biology Research Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486, **Department of Pediatrics, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania 19104, and the ***Pediatric Medical Associates, Havertown, Pennsylvania 19083

Respiratory syncytial virus is recognized as the single most important viral agent causing serious respiratory disease in infants and young children (1–5). There is no currently acceptable vaccine against respiratory syncytial virus infection. Killed virus vaccines have not presented evidence of inducing high-level immunity (6–10) and live attenuated virus vaccines (11–15) given directly into the respiratory tract have not been satisfactorily developed to date.

A recent report from this laboratory (16) described the development and clinical testing of a new live attenuated respiratory syncytial virus vaccine that is unlike previously developed vaccines against the agent in that it is administered by parenteral injection. It causes minimal, if any, clinical reactions. The vaccine was originally reported to induce homologous neutralizing antibodies in 75–93% of initially seronegative persons. Since the time of publication, it was found that neutralization of respiratory syncytial virus by homologous human antibody is a serum complement-dependent event. When the same sera were reassayed for neutralizing antibody in the presence of added complement, nearly all initially seronegative persons were shown to have developed neutralizing antibody.

The present report describes the findings in the previous and in additional clinical trials in which all the sera from the vaccinated persons were assayed in the presence of added complement. The findings in tests to measure the distribution of neutralizing antibody against respiratory syncytial virus in children, according to age, is also presented. Finally, reported respiratory disease events that occurred in children during one year after vaccination are recorded.

Materials and methods. Preparation of the vaccine, viral infectivity titrations, serum neutralization test methods and clinical study procedures for studies 456 and 487 were given in detail previously (16). In the studies, infants and young children residing in suburban Philadelphia were each given 0.5 ml of vaccine subcutaneously. Older siblings or close contacts served as controls. Clinical reactions were observed for 28 days after vaccination and blood samples were collected immediately prior to and 6 weeks after vaccination to measure antibody responses and to detect possible spread of the infection to susceptible contacts. Additional clinical studies numbers 508, 509, and 521 were carried out in the same way in the same open populations in the Havertown area of suburban Philadelphia. Modification of the serum neutralization test in the present study consisted of adding sterile guinea pig complement (Flow Laboratories, Rockville, MD) in a final concentration of 10% to the virus-human serum mixtures prior to the one hour incubation at 37°.

Results. Potentiation of antibody titers by added complement. Table I shows the findings in representative tests of individual sera from nine children who were initially seronegative and who received respiratory syncytial virus vaccine. The examples were selected to represent the typical findings. Addition of fresh guinea pig complement increased the neutralizing antibody titers of the individual sera from 2- to 32-fold or more showing that the neutralization of respiratory syncytial virus by human antibody is a complement-dependent event. The comparative findings in tests of postvaccination sera from 78 initially seronegative children are shown in Table II. These results show the marked increase in titer of antibody that occurs when complement is added. Such increase occurred in all but a few sera and the mean fold-increase

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was approximately fivefold.

Neutralizing antibody responses to vaccination in initially seronegative children. Table III presents the findings in five clinical studies to measure the neutralizing antibody responses in 116 initially seronegative children to vaccination with lot 592 or 594 of respiratory syncytial virus vaccine. There were 13 susceptible sibling contact controls. The findings in studies 456 and 487, reported previously (16), presented data using serum neutralization tests that did not include added complement. In these and additional clinical studies in which all sera were tested with added complement, 113 of 116 children developed homologous neutralizing antibody following vaccination and the mean antibody titers ranged from 1:5 to 1:17. None of the 13 susceptible sibling or close contact controls developed antibody. Nasal secretions were obtained from a total of eight vaccinated children and tested for homologous neutralizing antibody by the method described earlier (16) but with added complement. Table IV shows that only one of four initially seronegative children showed the presence of antibody in his nasal secretions even though all four children developed circulating antibody. Three of four initially seropositive persons showed an increase in nasal antibody even though there was no apparent increase in circulating antibody in two of the three responders.

Neutralizing antibody responses in initially seropositive children given the vaccine. Sera from 52 initially seropositive children two months to three years of age were selected to test for neutralizing antibody, in the presence of added complement, in the sera before and after vaccination. The selection was made to achieve as equitable distribution according to age as was possible. Among the group, 15

TABLE I. POTENTIATION OF RESPIRATORY SYNCYTIAL NEUTRALIZING ANTIBODY TITER BY GUINEA PIG COMPLEMENT OF SERA FROM INDIVIDUAL VACCINATED PERSONS WHO WERE INITIALLY WITHOUT DETECTABLE ANTIBODY.

Subject No.	Neutralizing antibody titer following vaccination: Without added complement	With added complement	Fold-difference
24	2	4	2
1	2	8	4
27	4	16	4
36	8	32	4
13	2	16	8
10	2	16	8
22	16	128	8
3	<2	16	16 or >
17	<2	32	32 or >

TABLE II. COMPARISON OF POSTVACCINATION NEUTRALIZING ANTIBODY TITERS IN TESTS WITH AND WITHOUT ADDED COMPLEMENT AMONG 78 CHILDREN WHO WERE WITHOUT ANTIBODY[a] BEFORE VACCINATION.

Addition of complement	Numbers of postvaccination children, according to titer: <2	2	4	8	16	32	64	128 or >	Geometric mean titer
no	23	22	15	5	8	1	4	0	3
yes	1	4	11	10	22	16	8	6	16

[a] As assayed in the presence of complement.

TABLE III. SERUM NEUTRALIZING ANTIBODY RESPONSES IN INITIALLY SERONEGATIVE CHILDREN WHO RECEIVED RESPIRATORY SYNCYTIAL VIRUS VACCINE OR WERE CONTACT CONTROLS.

Study No.	Vaccinated persons: Vaccine: Lot	Vaccine: Passage No.	Age	Response: No. positive/total	Response: Mean[b] titer	Non-vaccinated controls[a]: Age	Response No. positive/total
456	592	10	7 mo–19 mo	7/7	13	22 mo	0/1
508	592	10	6 mo–3 yr	64/65	17		
509	592	10	8 mo–21mo	6/6	10	2–3 yr	0/5
521	592	10	6 mo–20 mo	14/16	7		
521	594	5	7 mo–12 mo	10/10	5		
487	594	5	9 mo–2 yr	12/12	15	16 mo–3 yr	0/7
			Total group	113/116 (97%)			0/13 (0%)

[a] Contact controls.
[b] Geometric mean titer.

TABLE IV. CIRCULATING AND NASAL NEUTRALIZING ANTIBODY IN CHILDREN[a] GIVEN RESPIRATORY SYNCYTIAL VIRUS VACCINE.

Subject No.	Neutralizing antibody titers, according to time of vaccination			
	Circulating		Nasal	
	Before	After[b]	Before	After[b]
31	<2	8	<2	<2
27	<2	16	<2	<2
36	<2	32	<2	<2
37	<2	128 or >	<2	4
25	4	64	<2	2
29	64 or >	128	<2	4
38	64 or >	256	<2	8
33	64 or >	128	4	4

[a] Age 9–21 months.
[b] Second samples were taken 6 weeks after vaccination.

TABLE V. SERUM NEUTRALIZING ANTIBODY RESPONSES IN INITIALLY SEROPOSITIVE CHILDREN WHO RECEIVED RESPIRATORY SYNCYTIAL VIRUS VACCINE (AGE 2 MONTHS TO 3 YEARS).

Initial titer	No. with fourfold or greater increase[a]/total
2	0/10
4	2/14
8	0/9
16	0/1
32	1/5
64	0/1
128	1/6
256	0/3
512	0/1
2048	0/1
8196	0/1
Total	4[b]/52 (8%)

[a] The responses were: 4 to 16 (7 months old); 4 to 64 (10 months old); 32 to 128 (19 months old); 128 to 2048 (11 months old).
[b] None of the 4 responders was less than 7 months of age.

children were 2 to 6 months of age and 37 were 7 to 36 months of age. Table V shows that only four of the total group had a significant elevation in antibody, indicating that booster responses, in the presence of preexisting antibody, are an infrequent event. The increases in antibody that did occur were all found in children seven months of age or older, an age at which maternal antibody against respiratory syncytial virus is rarely present.

Clinical reactions in the subjects. It was reported previously, for studies 456 and 487, that recipients of the vaccine showed no indication of illness caused by the vaccine and that if, indeed, such did occur, it would have been very mild and clinically inconsequential. Similar results, not reported in detail here, were obtained in studies 508, 509, and 521.

Clinical follow-up in vaccinated persons. Earlier testing by others (8–10) of a killed respiratory syncytial virus vaccine suggested that vaccination might actually have increased the severity of the disease upon subsequent natural infection with the virus. Surveillance has been carried out among the 392 persons vaccinated to date in which parents and their physicians reported respiratory episodes of significance that occurred among the vaccinated children during a one-year period following vaccination. Table VI shows that 22 respiratory illness episodes were reported. Fourteen of the illnesses occurred in children that were initially seropositive (5%) and 8 of the illnesses occurred in children that were initially seronegative (7%). Two of the children, one initially seropositive and one initially seronegative, showed two episodes of illness each. Eight of the total group were hospitalized for respiratory illness. Five of them were initially seropositive and three were initially seronegative. There was no indication of increased rate or severity of respiratory illness in children who had developed antibody to respiratory syncytial virus as a result of vaccination. Serum samples were collected from all the 22 cases prior to onset of illness and again at a later time period. The time interval between samples was too long in most instances to establish definitive diagnoses for any particular respiratory disease episode in the subjects. However, four instances of significant increase

TABLE VI. RESPIRATORY ILLNESS EPISODES REPORTED WITHIN ONE YEAR AFTER RESPIRATORY SYNCYTIAL VIRUS VACCINATION.

Diagnosis	Antibody status before vaccination	
	Positive (276 persons)	Negative (116 persons)
Pneumonitis	1	0
Bronchiolitis	8	1
Bronchitis	3	1
Laryngotracheobronchitis	1	4
Upper respiratory illness	1	2
Total	14 (5%)	8 (7%)

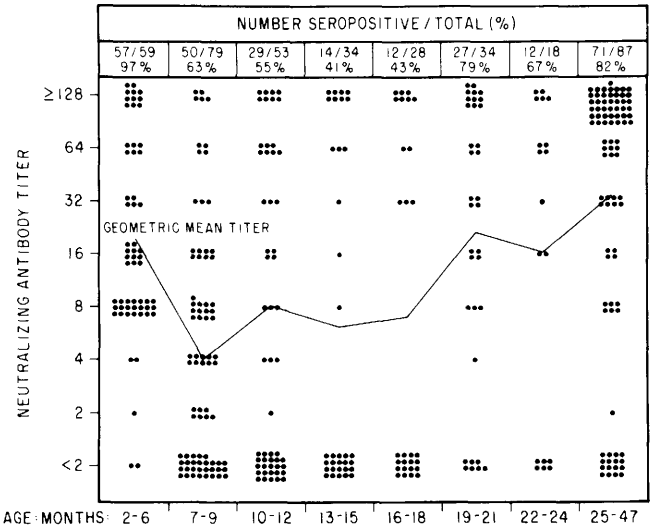

FIG. 1. Distribution in children, according to age, of neutralizing antibody against respiratory syncytial virus.

(fourfold or greater) in respiratory syncytial virus neutralizing antibody were found (two initially seronegative and two seropositive), showing that respiratory syncytial virus infections were indeed present in the test population. These cases were not confirmed in virus recovery attempts. There was no meaningful nonvaccinated control group for comparative purpose since the numbers were small and the control children were on the average older than those who were vaccinated.

Distribution of antibody according to age. All the initial serum samples from the 392 persons in the studies were retested for neutralizing antibody against respiratory syncytial virus in the presence of complement with the findings shown in Fig. 1. Following decline in maternal antibody during the first several months of life, there was progressive increase in seropositives with increasing age. It is of importance, however, that 118 of 333 or 35% of the total group of persons who were 7 to 47 months of age were still seronegative and only two infants who were less than 7 months of age were seronegative. Both of the two infants were 6 months of age.

Discussion. The findings in the present study show that neutralization of respiratory syncytial virus by homologous human antibody is a complement-dependent event. Complement derived from freshly collected guinea pig serum was capable of providing the neutralization enhancing factor. The consequence of adding complement was to increase the sensitivity of the assay, thereby increasing the antibody titers of the sera and the percentage of individuals for whom the presence of respiratory syncytial antibody could be detected.

Application of the more sensitive assay, in the present study, permitted more precise separation of the children who were seronegative prior to vaccination from those who were seropositive and gave a more accurate appraisal of the percentage of children who responded to vaccination. In the five studies carried out, there was a total of 116 initially seronegative children who were vaccinated and from whom paired serum samples were taken. Of these, 113 or 97% responded to the vaccine with the development of neutralizing antibody. The mean titers were increased in the new assay and ranged from 1:5 to 1:17. These antibody responses were remarkably similar numerically to those following vaccination with another member of the Paramyxovirus group (1) given parenterally, viz., mumps virus vaccine (17). Importantly, none of the 13 initially seronegative contact controls developed antibody indicating that contagious spread of the vaccine virus, if it ever occurs, must be of low frequency. These findings are consistent with those reported previously (16) in which none of 16 initially seronegative subjects given the vaccine excreted detectable respiratory syncytial virus in their respiratory secretions.

As expected, few increases in antibody oc-

curred in vaccine recipients who were initially seropositive (4/52 or 8%). All responses were in persons 7 months of age or older who might have been expected to have lost all their maternal antibody. Since circulating antibody in early life appears to be highly effective in neutralizing the virus and in precluding an active antibody response, it appears that the few initially seropositive people who did respond serologically to vaccination might have done so as a result of a booster response to the very small amount of antigen present in the vaccine. Such booster antibody effect might also have occurred in the three or four initially seropositive children who showed presence of antibody in their nasal secretions after vaccination. Only one of four initially seronegative children showed the development of nasal antibody in his nasal secretions after vaccination. The amount of protein in nasal secretions is very small and antibody is difficult to detect. Studies are being carried out to determine whether more efficient collection of protein from the nasal tract can be achieved, thereby providing more accurate measurement of the amount of nasal antibody. Nasal antibody is of importance in producing a barrier against infection with the virus in the respiratory tract.

The important matter of whether vaccination against the respiratory syncytial virus might actually potentiate the disease (8–10) on subsequent natural infection with the agent was at least partially answered in the present investigation. There were few reports of severe respiratory illness in any of the vaccinated persons and there was no greater incidence or severity in children who were initially seronegative than in those who were seropositive before vaccination.

In spite of early infection with respiratory syncytial virus, there is still a substantial portion of children who remain seronegative even up to 4 years of age. Such seronegative persons are those who should most benefit from the vaccine. The severe depression of immune response to vaccine by maternal antibody and the variable rate at which maternal antibody is lost make it difficult to describe an optimum age at which the vaccine might be given most beneficially. It does seem clear, however, that more than one dose will need be administered to achieve optimal results and that at least one dose would need be administered in very early life at the age when the clinical disease is most severe.

Summary. Further clinical tests were carried out of a live attenuated respiratory syncytial virus vaccine administered parenterally. Neutralization of respiratory syncytial virus by homologous human antibody was found to be a complement-dependent event and addition of guinea pig complement greatly increased antibody titers and the sensitivity of the test. Ninety-seven percent of 116 initially seronegative children developed antibody following vaccination and there was no evidence for contagious spread of the infection. Clinical reactions, if any, were mild and inconsequential. A small portion of initially seropositive persons appeared to develop antibody by booster effect and a portion of initially seronegative and seropositive persons developed detectable nasal antibody. Long-term follow-up failed to reveal any increase in severe respiratory disease in initially seronegative persons who were vaccinated. A very substantial reservoir of seronegative persons was found in the 7 to 47 month age group (35%) revealing a target population that could most benefit from being given the vaccine.

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