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Live Respiratory Syncytial Virus Vaccine Administered Parenterally (40112)

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Respiratory syncytial virus, a member of the Paramyxoviridae, Genus Pneumovirus, (1), is recognized as the single most important viral agent causing serious respiratory disease in infants and young children (1–5). The virus is an important cause for bronchitis, bronchopneumonia, and pneumonitis in young children and is a frequent cause of coryzal illness in older children and adults in whom lower respiratory tract infection may also occur. First infection with the virus, commonly associated with bronchiolitis and pneumonia, often occurs in early life and is sometimes fatal.

There is no currently acceptable vaccine against respiratory syncytial virus infections. Killed virus vaccines have not presented evidence of inducing high-level immunity (6–10). Live attenuated virus vaccines (11–15) given directly into the respiratory tract have not been satisfactorily developed to date.

The present report describes the development and clinical testing of a new and unique live attenuated respiratory syncytial virus vaccine that is administered by parenteral injection. Infants and young children who are initially without neutralizing antibody against the virus develop such antibody following vaccination. The vaccine causes minimal, if any, clinical reaction.

Materials and methods. Vaccine. A throat swab taken January 26, 1973 from a patient (S.A., C-73-287) with respiratory syncytial virus infection was obtained on May 23, 1973 from Dr. Robert Chanock. An isolate, identified as strain 287 respiratory syncytial virus, was recovered in cell cultures of grivet monkey kidney that were prescreened to rule out presence of adventitious agents. One additional passage was made in grivet monkey kidney cells and subsequent passages were made in WI-38 line diploid human embryonic lung cell cultures that were monitored in accordance with the "Revised Standards for Karyology of Human Diploid Cell Cultures" (16). Virus that was propagated in fifth and in tenth WI-38 cell culture passage was used to prepare Lots 594 and 592, respectively, of vaccine. Virus-infected cell culture fluids were clarified by passing through a medium (15 micron) sintered glass filter, dispensed in 1.2 ml amounts into vials, and lyophilized. Appropriate *in vivo* and *in vitro* tests, including tests in monkeys, were carried out on the pre-clarified virus samples, on the vaccine, and on uninoculated control culture fluids to rule out the presence of extraneous microbial and viral contaminants by procedures that are consistent with current standards for safety of live virus vaccines (17). Electron microscopic examination of the vaccine using a thin sectioning technique (18) revealed 10^7–10^8 viral particles per ml. There was insufficient viral antigen in the vaccine to permit detection in the complement-fixation test (1:2). *Titrations* of viral infectivity were carried out in Hep-2 cells read 14 days after inoculation. The infectivity titer of passage 5, Lot 594, vaccine was $10^{-2.5}$/0.1 ml and that for passage 10, Lot 592 vaccine was $10^{-2.6}$/0.1 ml. *Serum neutralization* tests were performed using Hep-2 cell cultures. Strain 287 of respiratory syncytial virus seed stock was diluted to contain 10–30 $TCID_{50}$ per 0.1 ml and was mixed with equal volumes of serial twofold dilutions of subject sera that were inactivated by heating at 56°C for 30 min. Duplicate cell cultures were inoculated with 0.2 ml of the mixtures following incubation at 37°C for 1 hr and incubated at 37°C for 7 days at which time observations were made for cytopathology. The serum titer was the highest initial dilution of serum that totally prevented viral cytopathology. Titrations of the viral seed stock were carried out simultaneously to establish the $TCID_{50}$ dose. Cytopathology first

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appeared on the fifth day in cultures inoculated with 10–30 $TCID_{50}$ of virus.

Clinical populations. Two studies were carried out among infants and children from middle-class socioeconomic background who were in good health and who resided in open populations in the Havertown area of suburban Philadelphia. The studies were done in pediatricians' offices and informed written consents were obtained from the parents or guardians. *Study 487* of Lot 594 (passage 5) vaccine was carried out between October 12, 1976 and April 26, 1977 among 39 infants and children 9 months to 4 years of age. Twenty-one participants received 0.5 ml of vaccine subcutaneously and 18 older siblings or close contacts served as unvaccinated contact controls. *Study 456* of Lot 592 (passage 10) vaccine was carried out between April 7 and May 19, 1976 among 27 infants and children, 6 months to 3 years of age. Fifteen participants received 0.5 ml of vaccine subcutaneously. Twelve older siblings served as contact controls. Temperatures were taken daily by the mothers for 28 days, and all illnesses were reported to one of us (REW). Nurses made telephone calls twice a week and a home visit on day 5 or 6 to observe all children and encourage close observation, recording and reporting by the parent. Any child who became ill was cared for by his physician. Blood samples were taken from all the children immediately prior to and 6 weeks after the vaccine was given in order to measure antibody responses to the vaccine and detect possible spread of infection to susceptible contact controls. Nasal washings, free of blood, were collected from a portion of the subjects in Study 487 to test for nasal antibody response following vaccination. The samples were taken in 5 ml of saline solution instilled nasally.

Results. Neutralizing antibody responses to vaccination in initially seronegative children. Table I shows the homologous antibody titers 6 weeks after vaccination among children who received passage 5 or 10 respiratory syncytial virus vaccine or who served as unvaccinated controls. Ninety-three percent of passage 5 and 75% of passage 10 vaccine recipients developed antibody following vaccination and none of the 11 contact controls developed such antibody. Two of six persons tested who received passage 5 vaccine displayed detectable neutralizing antibody in the nasal secretions and none of the three initially seronegative contact controls developed antibody. The protein content of the nasal secretions ranged from 7.8 to 104.0 mg/100 ml. Not all secretions contained sufficient protein to permit detection of antibody, but the protein content of the two samples from each of the two patients in whom responses were noted were roughly the same (patient 25, 73.2 and 104.0 mg; patient 37, 36.4 and 49.7 mg).

Tests for neutralizing antibody responses in 14 initially seropositive children given the vaccine. As shown in Table II, 2 initially seropositive children (Nos. 18 and 24) showed fourfold or greater increases in antibody following vaccination, three were indeterminate, and nine showed no change.

Clinical reactions among the subjects. As might be expected, many of the children displayed episodes of respiratory illness during the 28-day observation period following vaccination. Table III summarizes the composition of the test and control groups according to age and number, and the numbers of respiratory disease episodes that were observed. All illnesses were mild, limited to the upper respiratory tract, and were diagnosed as colds or pharyngitis. No child displayed lower respiratory tract involvement. The distribution of such illnesses was not remarkably different between the groups whether initially seronegative, seropositive, and whether given vaccine or not. There was no indication of illness caused by the vaccine. If, indeed, such did occur it would have been very mild and clinically inconsequential.

Table IV shows the occurrence of fever, according to time after vaccination, among the initially seronegative and seropositive children who received the vaccine and among the unvaccinated controls. The occurrence of fever appeared to be randomly distributed among all the groups. Fever caused by the vaccine was infrequent, if it occurred at all.

Distribution of antibody, according to age. All the initial serum samples taken from the 66 subjects in the study were tested for presence and amount of neutralizing antibody against respiratory syncytial virus with the findings shown in Fig. 1. Among the subjects, there was gradual increase in percentage of

TABLE I. HOMOLOGOUS NEUTRALIZING ANTIBODY RESPONSES IN YOUNG INITIALLY SERONEGATIVE CHILDREN GIVEN LIVE RESPIRATORY SYNCYTIAL VIRUS VACCINE BY THE SUBCUTANEOUS ROUTE.

Vaccine		Patients			Neutralizing antibody response: Circulating			
						Seroconversion		Nasal
Lot No.	Passage No.	Group	Ident. No.	Age (Mos.)	Titer	No. Total	Rate	Titer
594 (C-D495)	5	*Vaccine*				13/14	93%	
			7	9	<2			
(Study 487)			31	9	4			<2
			24	10	4			
			25	10	4			2
			27	13	4			<2
			36	13	8			<2
			1	14	2			
			19	14	2			
			11	15	8			
			37	15	16			4
			5	16	4			
			18	18	32			
			13	20	2			
			22	32	16			<2
					Mean (4.9)			
		Control				0/8	0%	
			35, 30, 32, 20, 2, 12, 17, 8	16, 20, 20, 25, 28, 31, 43, 46	<2 <2 <2			<2, <2, <2
592 (C-D478)	10	*Vaccine*				6/8	75%	
			4	7	<2			
(Study 456)			13	7	2			
			8	11	<2			
			20	11	16			
			22	12	2			
			12	15	16			
			23	18	8			
			14	19	2			
					Mean (3.4)			
		Control						
			3, 7, 11	22, 28, 33	<2	0/3	0%	

seropositives with increasing age. Of the total group of persons, who were in the 6 to 48 months of age range, half were seronegative.

Discussion. The frequently severe and sometimes fatal respiratory illnesses in young children caused by respiratory syncytial virus infection justifies concerted effort to develop an effective vaccine. Reinfection that occurs in older children and adults is usually far less severe than in infants and manifestations of the illness are ordinarily limited to the upper respiratory tract, though bronchitis, bronchopneumonia or pneumonia may occur (1, 2, 11). The fact that frequency and severity of illness is inversely related to presence of circulating homologous neutralizing antibody (19, 21, 22) provides a basis for preventing or modifying the natural disease by vaccines.

Killed respiratory syncytial virus vaccines, though capable of stimulating antibody, have not shown great promise for preventing infection or illness caused by the virus. Infants given one particular killed vaccine (8–10) developed more serious illness than those who were not vaccinated. In attempts to explain this phenomenon, Chanock and coworkers (20) suggested that an immunopathologic reaction might be involved in the lungs of the children, on natural infection, that is due to absence of IgA antibody and the presence of circulating neutralizing antibody of the IgG class induced by killed vaccine or

passively transferred from the mother. Validity for this hypothesis is doubtful since recent studies have shown a relative sparing effect (21) from the disease in children less than 1 month of age when maternally acquired IgG antibody is at its highest level and since the severity of pneumonias caused by respiratory syncytial virus in early infancy is inversely related to the level of circulating neutralizing antibody (19, 21, 22). The more severe illness that occurred in infants who were given killed virus vaccine might have been due to the relative impurity of the vaccine used that might have induced sensitization to the tissue components in the vaccine or to tissue–viral antigen combinations.

TABLE II. NEUTRALIZING ANTIBODY FINDINGS IN INITIALLY SEROPOSITIVE CHILDREN WHO WERE VACCINATED.

Vaccine	Subject No.	Serum neutralizing antibody titer: Before vaccination	Serum neutralizing antibody titer: After vaccination
Lot 594	3	16	16
(pas. 5 vaccine)	28	32	32
	9	64	64
	15	64	64
	29	≥64	128
	33	≥64	128
	38	≥64	256
Lot 592	16	2	2
(pas. 10 vaccine)	10	32	32
	18	64	256
	6	128	128
	24	128	1024
	26	128	128
	2	1024	1024

Two live respiratory virus vaccines developed by Chanock *et al.* (11, 12, 15) that are administered by nasal inoculation have been tested in man. *RS-A2* (RSV-26°) vaccine (11, 13, 15), made from virus adapted to growth at low temperature, displayed limited infectivity for adults, was poorly immunogenic in infants and young children who were having first experience with the virus, and was not considered suitable for vaccination purpose. A second vaccine, prepared from a temperature-sensitive mutant of respiratory syncytial virus (ts-1) (12, 13, 14), was also considered unsuitable for routine use in young children because it produced illness in susceptible individuals, produced infections in which virus was shed that were contagious to susceptible contacts, and showed partial genetic reversion to "wild-type" genotype (23) following human infection. All of a group of initially seronegative children less than 6 years of age and about half of children 6–13 years of age developed neutralizing antibody. There was no evidence for potentiation of natural illness by the live attenuated virus vaccine (14).

The respiratory syncytial virus vaccine described in this report is novel in that it was administered parenterally and used the same route of administration as for live measles, mumps, and rubella virus vaccines. Most importantly, 19 of the 22 initially seronegative individuals (86%) developed circulating antibody and 2 of 6 of these persons who were tested developed detectable amounts of anti-

TABLE III. RESPIRATORY DISEASE EPISODES,[a] ACCORDING TO INITIAL SEROSTATUS AMONG VACCINEES AND CONTROLS.

Test group	Day of onset of illness	Pas. 5 vaccine (Lot 594): No. in group (age in mos.)	Pas. 5 vaccine (Lot 594): No. with illness (%)	Pas. 10 vaccine (Lot 592): No. in group (age in mos.)	Pas. 10 vaccine (Lot 592): No. with illness (%)
Vaccinated					
Initial seronegative	0–2	14 (9–32)	3 (21)	8 (7–19)	2 (25)
	3–14		6 (43)		4 (50)
	15–21		5 (36)		2 (25)
Initial seropositive	0–2	7 (9–21)	0 (0)	7 (6–19)	1 (14)
	3–14		5 (71)		2 (29)
	15–21		2 (29)		1 (14)
Unvaccinated controls					
All persons	0–2	18 (16–48)	2 (11)	12 (22–44)	1 (9)
	3–14		7 (39)		1 (9)
	15–21		5 (28)		1 (9)

[a] All were cases of upper respiratory illness.

TABLE IV. MAXIMUM TEMPERATURE, ORAL (°F), ACCORDING TO INITIAL SEROSTATUS, AMONG VACCINEES AND CONTROLS (SEE TABLE III).

Test group	Time period after vaccination (days)	Maximum temperature—No. (%)						
		Vaccine pas. 5 (Lot 594)				Vaccine pas. 10 (Lot 592)		
		<99	99–100.9	101–102.4	104.0	<99	99–100.9	101–102.0
Vaccinated								
Initial seronegative	0–2	4 (28.6)	9 (64.3)	1 (7.1)		3 (37.5)	5 (62.5)	
	3–14	4 (28.6)	8 (57.1)	2 (14.3)		1 (12.5)	6 (75.0)	1 (12.5)
	15–21	5 (35.7)	8 (57.1)	1 (7.1)		5 (62.5)	3 (37.5)	
Initial seropositive	0–2	4 (57.1)	3 (42.9)			3 (42.9)	4 (57.1)	
	3–14	4 (57.1)	2 (28.6)	1 (14.3)		2 (28.6)	5 (71.4)	
	15–21	5 (71.4)	1 (14.3)	1 (14.3)		2 (28.6)	5 (71.4)	
Unvaccinated controls								
All persons	0–2	11 (61.1)	6 (33.3)	1 (5.6)		3 (25.0)	9 (75.0)	
	3–14	7 (38.9)	9 (50.0)	2 (11.1)		2 (16.7)	10 (83.3)	
	15–21	7 (38.9)	10 (55.6)		1 (5.6)	7 (58.3)	4 (33.3)	1 (8.3)

body in the nasal secretions as well. As with mumps vaccine (24), the circulating neutralizing antibody titers obtained were far less (mumps mean about 8) than found following natural infection (mumps mean about 40), and few persons with preexisting antibody showed antibody increase on vaccination. Though respiratory syncytial virus is not ordinarily associated with systemic infection and viremia, there appeared to be sufficient replication following parenteral injection to permit development of a substantial circulating antibody response that was also found in the nasal secretions. Importantly, none of the vaccinated susceptible persons showed more than very minor respiratory illness, if any at all. None of the susceptible contacts developed antibody against the virus, indicating lack of contagious spread from the vaccinated persons. The vaccine, as presently constituted, presents very desirable attributes for a live virus vaccine and warrants continued studies to determine the efficacy in preventing illness caused by this agent. Importantly, the vaccinated subjects are being followed for clinical response on reinfection in nature with respiratory syncytial virus.

The question *might* reasonably be asked whether there was actual replication of the virus in the human subjects or whether the antibody response was due to presence of nonreplicating viral substance in the vaccine. Viral replication appears highly likely because of the extremely small quantity of virus in the vaccine (10^7–10^8 particles per ml and less-than-detectable amount of complement-fixing antigen), about 100-fold smaller in amount than the antigen mass needed, in our experience, to induce antibody in man for other viruses. For example, about 10^{11} particles of killed influenza viral antigen are needed per dose. Attempts, to date, not reported here, to isolate virus from the respiratory secretions of human subjects have all been negative. Respiratory secretions taken from 16 susceptible individuals at 3–5 day intervals for 21 days after vaccination were free of detectable respiratory syncytial virus. This is not surprising since virus also cannot be recovered from the respiratory secretions of subjects given measles or mumps vaccine.

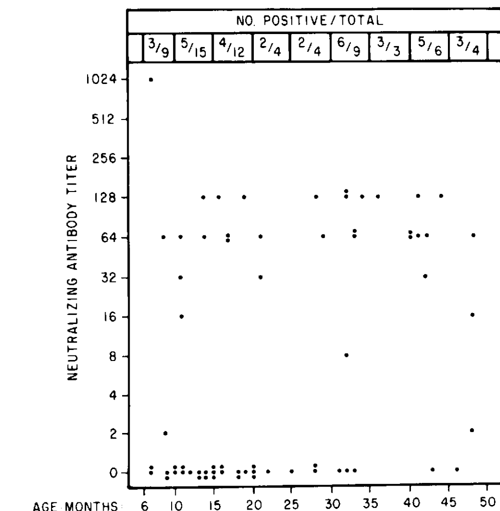

FIG. 1. Distribution in children, according to age, of neutralizing antibody against respiratory syncytial virus.

Attempts to detect viremia in vaccinated subjects have not been carried out to date since the question does not appear to justify the need for repeat venipunctures in young children.

The question might also be asked as to what persons might benefit most by use of live respiratory syncytial virus vaccine. Clues are provided by data relating to the natural experience with the virus. Chanock *et al.* (25) noted that essentially all infants are born with serum neutralizing antibody of maternal origin and that such antibody decreases in titer approximately twofold per month. First infection with respiratory syncytial virus typically occurs within the first few years of life. Community-wide epidemics of the infection may occur every year during the fall, winter, and spring seasons (25). As noted above, neutralizing antibody that arises following infection is of long-term persistence and is associated with illness of reduced severity when reinfection occurs. Antibody of maternal origin appears to afford substantial protection in early life, and this is substituted by actively acquired antibody that is reinforced by repeated infections thereafter. Reinfection by viruses, in spite of circulating antibody, is a common event, e.g., poliomyelitis, measles, mumps, and rubella. Infections that depend, for illness, upon systemic distribution are usually clinically inapparent on reinfection (measles, mumps, rubella, poliomyelitis) while repeat infections that are more superficial (influenza, respiratory syncytial virus) commonly cause illness, though less severe.

It would be desirable to acquire active immunity against respiratory syncytial virus in infancy and early childhood by use of a vaccine that causes no apparent clinical reaction. In the open populations investigated in the present study, only half of the 66 persons, 6–48 months of age, had demonstrable antibody against the virus. In such a population, the vaccine might have special usefulness during early infancy and in the preschool years as well. Immunization in the early postnatal period, before 6 months of age, would be complicated by the unpredictable presence in some individuals of neutralizing antibody that suppresses an active immune response. Administration of more than a single dose of the vaccine might be required to obtain maximal benefit from use of the vaccine. The importance of the disease caused by respiratory syncytial virus, the deficiency of antibody among children who might benefit from vaccination, and the ability of the parenterally administered live virus vaccine to induce antibody without causing illness justify further study of the present vaccine to measure its protective efficacy and the optimal conditions for its use.

Respiratory syncytial virus is a member of the family Paramyxoviridae (1) that includes the genera Paramyxovirus (paramyxoviruses and mumps), Morbillivirus (measles–distemper), and Pneumovirus (respiratory syncytial virus). Parenterally administered live measles and mumps virus vaccines are highly satisfactory and are routinely administered. The present respiratory syncytial virus vaccine represents a third example of a vaccine of the family Paramyxoviridae given parenterally. In further studies (unpublished), we have employed the same approach to parenteral administration of live parainfluenza 1, 2, and 3 virus vaccines. Types 2 and 3 have shown considerable promise in human trials to date. Parainfluenza 1 virus has been of less promise to date, since it grows only poorly in any presently known cell culture medium suitable for use in man.

Summary. A unique live attenuated respiratory syncytial virus vaccine that is administered parenterally is described. Nineteen of 22 initially seronegative children, 7–32 months of age, developed homologous circulating neutralizing antibody against the virus and a portion were shown to have present demonstrable antibody in their nasal secretions. None of the children developed illness that could be ascribed to the vaccine, and there was no evident contagious spread of the vaccine virus to susceptible contacts. The vaccine is of considerable promise and trials to determine protective efficacy are being pursued. Seroepidemiologic investigations revealed that only 33 of 66 children 6–48 months of age and residing in an open population had detectable circulating neutralizing antibody against the virus.

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