AN EPIDEMIOLOGIC STUDY OF ALTERED CLINICAL REACTIVITY TO RESPIRATORY SYNCYTIAL (RS) VIRUS INFECTION IN CHILDREN PREVIOUSLY VACCINATED WITH AN INACTIVATED RS VIRUS VACCINE

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Kapikian, A. Z. (Lab. Infectious Diseases, N.I.A.I.D., N.I.H., Bethesda, Md. 20014), R. H. Mitchell, R. M. Chanock, R. A. Shvedoff and C. E. Stewart. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. Amer. J. Epid., 1969, 89: 405–421.—A formalin inactivated monkey kidney culture propagated 100-fold concentrated respiratory syncytial (RS) virus vaccine was administered intramuscularly to residents of Harrison and Arthur Cottages in Junior Village, a District of Columbia Welfare Institution for homeless but otherwise normal infants and children. No significant local or systemic vaccine reactions were observed. One to three doses of vaccine were found to produce CF antibody titers of 1:8 to 1:256 in 27 (97%) of 28 seronegative (<1:4) residents; the vaccine also stimulated high levels of neutralizing antibody. A sharp outbreak of RS virus infection occurred approximately 9 months after the vaccine study was initiated; RS virus was recovered from 60 (41%) of 146 residents and serologic evidence of infection was detected in 37 (92%) of 40 seronegative individuals. Recovery of RS virus was found to be significantly associated not only with the onset of febrile illness but also with the onset of febrile pneumonia illness. The vaccine not only failed to offer protection but also induced an exaggerated, altered clinical response to naturally occurring RS virus infection in the younger vaccinees as 9 (69%) of 13 vaccinated and only 4 (9%) of 47 nonvaccinated Harrison Cottage residents 6–23 months of age developed pneumonia (p < .0001). The paradoxical effect of vaccination suggests that serum antibody may play an active role in the pathogenesis of RS virus disease.

antibodies; epidemiology; immunity; pneumonia; respiratory syncytial virus; respiratory tract diseases; vaccines; viral vaccines

INTRODUCTION

RS virus has been shown to be the most important viral respiratory tract pathogen of infants and children, particularly during the first six months of life (1, 2). It recurs

in epidemic form yearly for a three to four month interval and is responsible for severe lower respiratory tract illnesses, notably bronchiolitis and bronchopneumonia in infants. It also causes pneumonitis, bronchitis, and febrile upper respiratory illnesses in infants and young children. Intensive efforts have been made to develop an antigenic RS virus vaccine in an attempt to control illnesses caused by this virus. Several previous studies have presented data indicating that even if such a vaccine could be developed, protection against illness might be difficult to achieve since 1) moderate levels of serum neutralizing antibody from previous natural infection did not protect against RS virus lower respiratory tract illness, and 2) RS virus associated bronchiolitis and pneumonia occurred more often in the first four months of life when maternally transmitted antibody was at its highest levels than during the later months of infancy when passive antibody levels had decreased markedly or disappeared (1–5). It was hoped, however, that an antigenic vaccine which could induce the development of quite high levels of serum antibody would be effective in preventing lower respiratory tract illness. This report describes an epidemiologic study in which an inactivated RS virus vaccine was administered to residents of Junior Village (J.V.), a District of Columbia Welfare Institution for homeless but otherwise normal children; the vaccine had been shown to be nontoxic and antigenic in adult volunteers and nontoxic in infants and children before the J.V. vaccine study was initiated (6, 7). The objectives of the Junior Village RS vaccine study were to determine (a) the nature of local and systemic reactions to the vaccine, if any, (b) its antigenicity, and (c) its protective efficacy during a

naturally occurring outbreak of RS virus infection and illness.

MATERIALS AND METHODS

Vaccine

The formalin inactivated RS vaccine was obtained from Pfizer Laboratories Inc., N.J., which prepared it under contract to the Vaccine Development Branch, N.I.A.I.D., N.I.H. The virus employed in the preparation of the vaccine was the Bernett strain isolated in 1961 in primary human embryonic kidney (HEK) tissue cultures from a throat washing taken from a volunteer previously inoculated with RS virus. It was passaged three additional times in primary HEK cultures, and ten times in vervet monkey kidney cultures. A 1:50 final dilution of seed virus was inoculated into vervet monkey kidney bottle cultures maintained with Eagles Basal medium in Earle's salt solution (EBME) without serum. Tissue culture fluid harvests were subsequently clarified by filtration, inactivated with formalin, concentrated by centrifugation and alum precipitation, supplemented with preservatives, tested for antigenicity in animals, and safety tested in animals and numerous culture systems. Details of these vaccine production procedures are presented in an accompanying report (8).

Plan of study

The study group was composed of the youngest and second youngest J.V. groups who lived in newly constructed separate cottages designated as Harrison and Arthur Cottages, respectively. Each cottage was divided into three adjoining wings (wings 1, 2, 3), each providing facilities for one third of its residents. A separate clinic adjoined Harrison Cottage and residents from Harrison, Arthur and Cleveland Cottages were examined there. New admissions from six months to five years of age were admitted to the special admissions unit (wing 3) in Harrison Cottage;

children usually resided there for three weeks before being transferred, according to age, to other wings of Harrison, Arthur and Cleveland Cottages. Harrison Cottage wings 1 and 2 housed children six months to approximately two years of age; Arthur Cottage housed children approximately two to four and a half years of age; and Cleveland Cottage housed those about four and a half years or older. Wing 1 of Arthur Cottage, which housed the oldest children in this cottage, was not included in the study because its residents frequently left the cottage for outside activities and consequently were not available for close observation. Therefore, children residing in three wings of Harrison and two wings of Arthur Cottage comprised the study group. As resident children became older, they were transferred to cottages housing children of the same age. On the average, approximately six children were admitted to Harrison Cottage and approximately two to Arthur Cottage each week, while between five and six were discharged from Harrison Cottage and between two and three from Arthur Cottage weekly. The mean weekly population for Harrison Cottage was approximately 52 while that for the two study wings of Arthur Cottage was about 45. The average duration of residence for children who were in residence at least one week in both cottages combined was approximately 13.5 weeks. Over 90 per cent of the residents were Negro.

The assignment of children into the vaccinated or nonvaccinated group was made according to the time and day of admission to Junior Village. Children were assigned numbers consecutively depending on admission time and those with odd numbers were selected to be vaccinees while those with even numbers were not vaccinated. The RS vaccine was packaged by the manufacturer in 3 ml vials. It was stored at 4 C and shaken well before inoculation. Five-tenths ml of vaccine was administered intramuscularly and similar injections were given approximately four and eight weeks

later by the physician in charge. This schedule could not always be followed because of intercurrent illnesses, discharge, or discharge with subsequent readmission when the vaccination schedule was resumed. The vaccinee's arm was examined daily by the nursing staff for about one week after the administration of vaccine. The first dose of vaccine was administered on February 28, 1966 while the last dose was given on December 20, 1966.

Since 1955, our laboratory has studied the infectious disease experience of the youngest group of children at Junior Village (9). A full time medical staff of two nurses and four practical nurses from our unit were in daily attendance, in addition to the full time medical staff of the District of Columbia Welfare Department, which included nurses, practical nurses, and a full time physician in charge of the medical care of residents of Harrison, Arthur and Cleveland Cottages. In Harrison Cottage a rectal temperature was obtained on each child once a day from the beginning of the vaccine study, while in Arthur Cottage routine daily temperatures were not taken until July 17, 1966. Routinely a physician examined any child with a rectal temperature of 100.6 F or greater. Children from both study cottages were examined in the clinic adjoining Harrison Cottage and those with moderately severe illnesses but not serious enough to require hospitalization were transferred from their respective cottage to an infirmary building which provided special care for ill children from all cottages. Children with more serious illnesses were transferred to District of Columbia General Hospital. The diagnosis of pneumonia or pneumonitis was made by the examining physician when he heard fine or medium crepitant inspiratory rales on auscultation. The day of onset for the pneumonia illness was considered to be that day when the child's temperature first reached 100.6 F or greater. The examining physician had access to information concerning a resident's vaccine status.

Collection of specimens

Throat swabs for virus culture were taken twice weekly, usually Monday and Thursday unless a holiday intervened. A dry cotton swab was streaked across the posterior pharynx and tonsillar pillars and then immersed in a vial containing 3 ml of veal infusion broth with 0.5 per cent bovine albumin and 250 units penicillin, 250 micrograms streptomycin, and 5 micrograms amphotericin per ml. The specimens were kept cold in wet ice and inoculated into tissue cultures within two hours of collection. Until the outbreak was recognized, only specimens obtained on Thursday were inoculated into tissue culture; however, beginning on December 27, 1966, specimens were tested twice weekly. Blood samples were obtained on admission, and at approximately three month intervals thereafter.

Virus isolation

Laboratory procedures were performed by individuals who did not have knowledge of the vaccine status of the children. Each of the following was inoculated with 0.2 ml of throat swab fluid: two roller tubes of Hep$_2$ tissue culture maintained in a 1:1 mixture of Eagles medium and Medium 199 with 5 per cent inactivated (56 C for 30 minutes) chick serum, two roller tubes of human diploid cell strain (HDCS) WI38 tissue culture maintained in a 1:1 mixture of Eagles medium and Medium 199 with 2 per cent inactivated fetal calf serum, and two roller tubes of rhesus monkey kidney tissue culture maintained in a 1:1 mixture of Eagles medium and Medium 199 with 0.2 per cent SV5 antiserum. After several cases of pneumonia had been recognized and after it was learned that RS virus was present in the Washington area, intensive measures for the isolation of RS virus were instituted on December 27, 1966 (6). Beginning on this date, Hep$_2$ cultures were obtained from three different sources and each specimen was inoculated into two Hep$_2$ tubes from

each source. This was done to minimize periodic variation in sensitivity of Hep$_2$ tissue for RS virus. This effort was necessary since the virus is recovered with difficulty from specimens which have been frozen and thawed (3, 11).

All tissue cultures were incubated on a roller drum and observed for cytopathic effect (CPE) two to three times weekly for approximately three weeks. If the characteristic syncytial cytopathic effect of RS virus in Hep$_2$ cells was observed, the fluid of the affected culture was inoculated into other Hep$_2$ cultures and in addition an aliquot was tested by complement fixation (microtiter test) with specific RS virus ferret serum. If the first harvest was negative or was anticomplementary an aliquot of fluid from the second passage harvest was tested by complement fixation (CF). The ferret serum employed in the CF test was prepared by infecting ferrets with the Long strain of virus; it fixed complement with 4 units of RS antigen at a dilution of 1:128 and did not react with normal uninfected Hep$_2$ antigen or any of the known myxoviruses or paramyxoviruses. An isolate was classified as RS virus if it produced the characteristic syncytial CPE in Hep$_2$ cultures and if it completely fixed complement with 4 units of ferret antiserum. In addition, all harvests yielding RS virus were also tested for presence of adenovirus and herpes simplex antigens by CF to determine if double infections had occurred.

The monkey kidney cells were tested for hemadsorption at approximately five day intervals and if hemadsorption occurred, hemadsorption-inhibition tests were performed. If CPE resembling herpes simplex or adenovirus developed in HDCS WI38 or Hep$_2$ cells, the fluid and cells were harvested and tested by CF with specific adenovirus and herpes simplex sera. In addition, if a CPE not resembling a picornavirus, adenovirus, or herpes simplex virus developed in HDCS WI38, the harvest was tested by CF for RS virus.

Serology

Neutralizing antibody was determined by the plaque reduction technique as described previously using a strain of RS virus isolated during the Junior Village outbreak (12). Three Petri dish cultures of Hep_2 cells were used for each serum dilution tested. The serum antibody titers were calculated on the basis of a 60 per cent plaque reduction endpoint by probit analysis. The serum titers were expressed as the initial serum dilution before addition of RS virus. RS virus CF antigen was prepared from the Long strain grown in Hep_2 culture; the A and B antigens of RS virus were prepared as previously described (13). The CF test was performed by a modification of the Bengston technique using microtiter plates, 1.7 units of complement and overnight incubation at 4 C (14, 15). Sixteen units of CF antigen were used in all tests except those in which the A and B antigens were used when 4 units were employed.

RESULTS

Vaccine reactions

The vaccinated and control groups were comparable in age, sex, and race. There was no significant difference in febrile illness rates between vaccinated and control groups within the first three days following inoculation. In addition, no significant immediate or delayed local reactions were observed.

Antigenicity of vaccine

As shown in Figure 1, 27 (96 per cent) of 28 infants and children who lacked detectable (<1:4) pre-vaccination CF antibody developed such antibody at a titer of 1:8 to 1:256 following one to three injec-

PRE AND POST VACCINATION CF ANTIBODY TITERS OF J.V. RESIDENTS
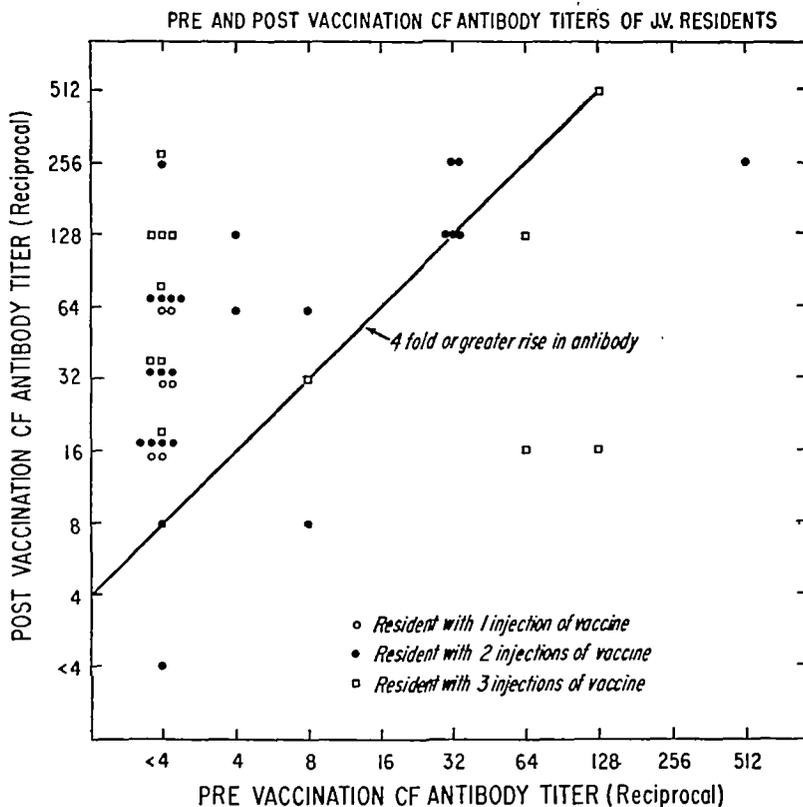
FIGURE 1

tions of vaccine. In addition, 10 (67 per cent) of 15 with prevaccine CF antibody developed 4-fold or greater increases after vaccination. The average age of the 43 vaccinated children shown in figure 1 at the time of last injection was 22.5 months while the median age was 19.5 months. Most of the post vaccination sera were obtained within four weeks of vaccination. Four vaccinees' pre- and post-vaccine sera were also tested by the CF technique using both an RS virus suspension and its A and B components in an attempt to determine the type of antibody induced by the vaccine. Table 1 shows that, following vaccination, CF antibody developed not only to the virus suspension antigens but also to both the A and B components of RS virus, a response similar to that found after natural infection (13).

In a limited survey, neutralizing antibody rises, as measured by 60 per cent plaque reduction of the J.V. outbreak RS virus strain were also demonstrable; each of seven vaccinees, four with two doses and three with three doses of vaccine, without detectable (<1:20) prevaccination anti-

TABLE 1

Reciprocal of complement fixing antibody titers of vaccinees to whole virus and A & B antigens of RS virus

No. of doses of vaccine given	Serum tested	CF antibody response to indicated antigen		
		RS virus infected tissue culture fluid*	A antigen of RS virus†	B antigen of RS virus†
2	Prevaccine	<4	<4	<4
	Postvaccine	256	256	8
2	Prevaccine	<4	<4	<4
	Postvaccine	32	32	4
	Postoutbreak	≥512	≥512	≥512
3	Prevaccine	<4	<4	<4
	Postvaccine	64	64	32
	Postoutbreak	≥1024	512	≥64
3	Prevaccine	<4	<4	<4
	Postvaccine	64	128	8
	Postoutbreak	128	128	32

* 16 units of antigen used.
† 4 units of antigen used.

body developed antibody titers ranging from 1:1000 to >1:10,000. In addition, 18 of 19 vaccinees, two with one dose, nine with two doses, and seven with three doses, with prevaccination titers of 1:25 to 1:2500 developed 4 to 400-fold rises in antibody levels following vaccination.

The outbreak

Except for occasional outbreaks, usually associated with specific agents, pneumonias have occurred only sporadically in the study group. However, in a 13-day period, December 16 to 28, 1966, in Arthur Cottage, and a 16-day period, December 28 to January 12, in Harrison Cottage, 24 children had an onset of a pneumonia illness; these children represented 14 per cent of the residents of Harrison Cottage and 21 per cent of those in Arthur Cottage (table 2). Only children in residence at least seven days between December 4, 1966, and January 31, 1967, were included in the analysis. The difference in population size of the two cottages was a result of the conversion before the outbreak period of one of the three wings in Harrison Cottage to an admissions unit for both cottages in an attempt to isolate new admissions who might be incubating illnesses; children usually resided in the admission wing for three weeks before being transferred to the other wings of Harrison or Arthur Cottages. In addition, one wing of Arthur Cottage, which housed the oldest children, was not included in the study since these children frequently left the cottage for outside activities and were not available for close daily observation.

RS virus was isolated from 41 per cent of the residents while 78 per cent of those tested by both virus recovery and serologic techniques demonstrated evidence of RS virus infection; in addition, 92 per cent of the residents without detectable preoutbreak CF antibody developed serologic evidence of RS virus infection (figure 2 and table 2). These findings indicate that RS

TABLE 2

Incidence of RS virus infection and pneumonia at Junior Village

Cottage	No. at risk*	Age (in months)			No. who developed pneumonia	RS virus infection as detected by:							
						Virus isolation		Complement fixing antibody rise				Virus Isolation and/or antibody rise	
								Total Group		Those without preoutbreak CF antibody†			
		Mean	Median	Range		No. Tested	No. Positive	No. Tested	No. Positive	No. Tested	No. Positive	No. Tested	No. Positive
Harrison	94	24	19	5–68	13 (14%)	94	41 (44%)	45	37 (82%)	29	28 (97%)	45	40 (89%)
Arthur	52	34	33	15–65	11 (21%)	52	19 (37%)	32	16 (50%)	11	9 (82%)	32	20 (63%)
Total	146	28	27	5–65	24 (16%)	146	60 (41%)	77	53 (69%)	40	37 (92%)	77	60 (78%)

* Includes only those infants and children who were in residence for 7 or more days during the outbreak period—Dec. 4, 1966, to Jan. 31, 1967.

† Preoutbreak CF antibody not detectable at a serum dilution of 1:4.

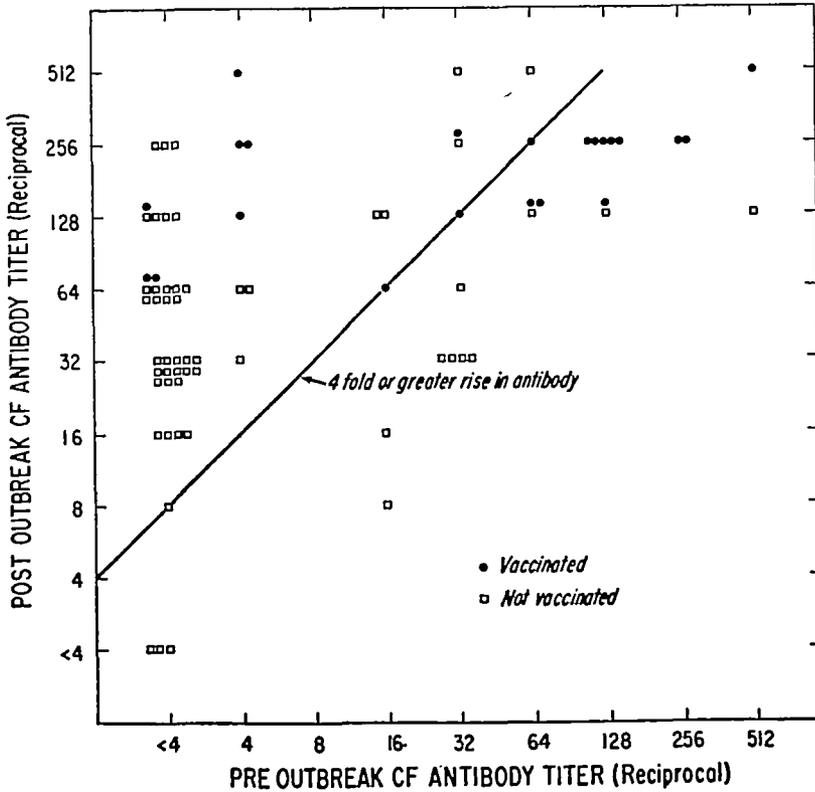PRE AND POST OUTBREAK CF ANTIBODY TITERS OF VACCINATED AND NONVACCINATED RESIDENTS

FIGURE 2

ONSETS OF PNEUMONIA IN STUDY GROUP BY DAY

HARRISON COTTAGE

ARTHUR COTTAGE

Number of initial isolations of RS virus in:															
Harrison Cottage	0	0	0	8	0	11	11	2	5	1	1	1	0	1
Arthur Cottage	0	0	1	14	4	0	0	0	0	0	0	0	0	0

↑ Satisfactory test for RS virus in Hep₂ tissue culture

■ Child in residence at start of outbreak period (Dec. 4, 1966)

☐ Child admitted to indicated cottage after onset of outbreak period. Numbers above squares indicate days from admission to indicated cottage to onset of pneumonia.

FIGURE 3

virus infection was extensive and involved most of the nursery residents.

Figure 3 shows the day of onset of pneumonia and the number of initial RS virus isolations on each test day during the outbreak period for each cottage. The number of RS virus isolations reached a peak on December 27 after intensive measures (as described in Materials and Methods) had been instituted for the recovery of this agent. All 60 initial RS virus isolations were made in Hep₂ cultures; no RS virus isolations were made in HDCS WI38 or rhesus monkey kidney cultures. For purposes of analysis the beginning of the epidemic period was arbitrarily set at December 4, 1966, since the first case of pneumonia occurred approximately two weeks after this date, and it is possible that if intensive measures had been instituted sooner, RS virus might have been recovered in the early December period. The end of the epidemic period was set at January 31, 1967, since the number of RS isolations had diminished markedly by this date and it appeared that most susceptibles had become infected. During the month of February only two isolations of RS virus were made, one from a child who de-

veloped pneumonia four days after the day of virus isolation.

The rapid spread of RS virus after its initial isolation is shown in figure 4. It was of interest that each of the initial RS virus isolations from children in residence in Harrison Cottage at the time virus was first detected was made within 20 days of this time; subsequently, virus was recovered only from children admitted after the time virus was first detected. A similar analysis of virus spread in Arthur Cottage could not be made since it appeared that intensive measures to isolate RS virus were not instituted until after the introduction of the virus into this cottage, and also since almost all new admissions to Arthur Cottage were initially admitted to the special admissions unit of Harrison Cottage.

Relationship of virus isolation to illness

To determine if RS virus was associated with disease, the febrile (≥100.6 F) illness attack rate during a five day period bracketing virus isolation (test period) among the 60 children who were RS virus positive was compared with the illness attack rate of these same children during an

ISOLATION OF RS VIRUS ACCORDING TO DAYS FOLLOWING
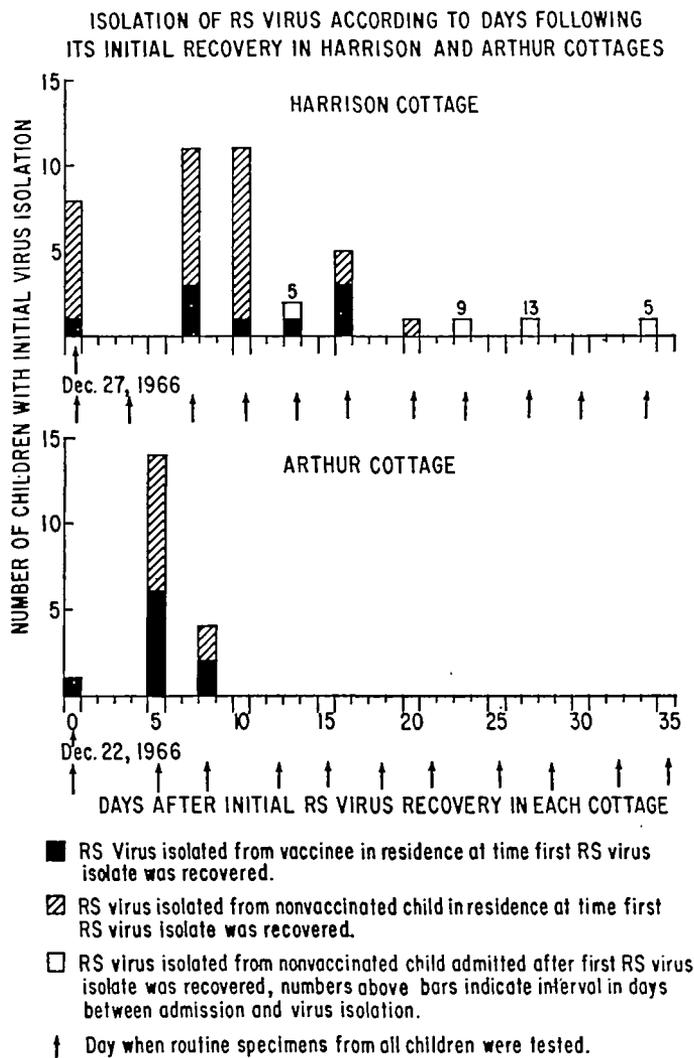ITS INITIAL RECOVERY IN HARRISON AND ARTHUR COTTAGES

FIGURE 4

11-day period immediately before and a 13-day period immediately after the test period (horizontal analysis) (3, 9). In such an analysis, there was no need to adjust for attributes such as age, sex, race, duration of residence, etc. since each child served as his or her own control. The five day period was chosen on the assumption that if the virus caused the illness, then it should have been recoverable at least one day before, the day of, or up to three days after the onset of the febrile episode. The adequacy of the test period was supported by a volunteer study which showed that of 20 adult volunteers who became ill after RS virus challenge, 12 shed the virus at least one day before and seven shed it for four days after onset of symptoms (16).

Using the horizontal analysis as described above, the data were first analyzed for association of RS virus infection and the most objective measure of illness, i.e., a febrile episode of 100.6 F or greater. Table 3 shows that the febrile illness onset rate was more than three times greater during the test period than during the 24 day control period, indicating an association between RS virus recovery and the

onset of a febrile illness in these children ($p < .0001$). A similar horizontal analysis was made to determine if RS virus infection was associated significantly with the onset of a febrile pneumonia illness, and it was found that an onset of a febrile pneumonia illness occurred during nine (4.2 per cent) of 215 child-days at risk during

the test period and only three (0.3 per cent) of 1030 child-days at risk during the control period. Thus, there was also a significant association between RS virus isolation and the onset of a febrile pneumonia illness ($p < .0001$).

Significance of serum neutralizing antibody

Suitable preoutbreak blood specimens were available from 85 of the 109 nonvaccinated residents. Table 4 shows that the prevalence of serum neutralizing antibody increased with age; 53 per cent of residents 6–23 months of age lacked detectable ($\geq 1:20$) preoutbreak antibody while only 12 per cent of children 24–65 months of age were seronegative. In addition, only 23 per cent of the nursery residents who were 6–23 months of age had a serum antibody titer of 1:401 or greater while 62 per cent of those 24–65 months of age had a similar level of antibody. In contrast all the vaccinees tested (29 of 37 had suitable preoutbreak blood specimens) possessed preoutbreak (postvaccine) antibody, and in comparison to the nonvaccinated group significantly more vaccinees had high (>1:1280) serum antibody titers ($p < .0001$). This difference undoubtedly reflects an antigenic effect of the vaccine.

TABLE 3

Febrile illness experience of 60 RS virus positive children during 214 child-days at risk in the test period compared with that of the same children during 1030 child-days at risk in the control period

Period†	Child-days at Risk*		
	With onset of febrile illness*	Without onset of febrile illness	Total
5 day period bracketing initial virus isolation (test period)	36 (17%)	179	215
11 day period before and 13 day period after test period (control period)	52 (5%)	978	1,030
Total	88 (7%)	1,157	1,245

$p < .0001$

* An onset of illness was considered to be the first day the temperature reached 100.6 F or greater and a child was considered to be at risk on the day of onset and on each day following an afebrile (<100.6 F) period of two days.

† The period is actually two weeks before and two weeks after initial RS isolation and is divided into test and control periods as described previously (3).

TABLE 4

Serum neutralizing antibody status of vaccinated and nonvaccinated residents of Junior Village just prior to onset of RS virus outbreak

Reciprocal of serum dilution which produced 60% reduction of RS virus plaques*	No. of nursery residents of indicated age in months†									
	Vaccinees					Nonvaccinated Group				
	6–11	12–23	24–35	36–58	Total	6–11‡	12–23	24–35	36–65	Total
<20					0	4 (57%)	19 (53%)	4 (15%)	1 (6%)	28 (33%)
20–100					0	2	5	3	1	11 (13%)
101–400		2	1	1	4 (14%)		3	5	2	10 (12%)
401–1,280	1		2	1	4 (14%)	1	5	8	5	19 (22%)
>1,280		6	6	9	21 (72%)		4	6	7	17 (20%)
Total	1	8	9	11	29	7	36	26	16	85

* Tested with RS virus strain recovered from Junior Village outbreak.
† Includes only individuals in residence at least 7 days during outbreak period, Dec. 4, 1966–Jan. 31, 1967.
‡ Includes one child aged 5 months.

The presence or absence of preoutbreak antibody did not appear to influence the occurrence of pneumonia among nonvaccinated children as 11 per cent of the residents who lacked preoutbreak antibody developed pneumonia while similar disease occurred in 14 per cent of children with prior antibody (table 5). Nonvaccinated children with preoutbreak antibody shed RS virus significantly less frequently than those without antibody as 15 (26 per cent) of 57 with a preoutbreak serum titer of 1:20 or greater and 21 (75 per cent) of 28 who were seronegative (<1:20) shed the virus ($p < .0001$) (table 5).

It appeared that residents without pre-existing antibody tended to shed the virus for a longer period than did seropositive children; in both cottages combined, six of 21 nonvaccinated RS virus positive children who lacked pre-existing antibody shed virus for four to 14 days while in Harrison Cottage only one of 10 nonvaccinated RS positive children with prior antibody shed virus for four days.

Clinical response of vaccinated and nonvaccinated groups to RS virus infection

We did not limit our analysis of vaccine efficacy to the vaccinees and their contemporary controls who were alternate admissions to J.V. during the period that vaccine was administered. As shown in table 2 almost all Harrison and Arthur residents who lacked CF antibody in preoutbreak sera were infected with RS virus if they were in residence at least one week during the RS outbreak. In a previous RS outbreak at Junior Village, almost all residents were infected if they were present for at least one week (3). Therefore, for analysis of vaccine efficacy, the control group was expanded to include those nonvaccinated individuals who were admitted to the nursery prior to or during the outbreak, as well as the controls who were alternated with the vaccinees during the time vaccine was given. The age distribution of the vaccinated and nonvaccinated groups was comparable.

TABLE 5

Relation of pre-existing serum neutralizing antibody to pneumonia and infection in nonvaccinated residents of Junior Village nursery during RS virus outbreak, Dec., 1966–Jan., 1967

Reciprocal of serum dilution which produced 60% reduction of RS virus plaques*	Total population: Each individual studied by virus recovery technique			Group studied by both virus recovery and CF antibody techniques	
	No. nursery residents†	No. who developed pneumonia	No. from whom virus recovered	No. nursery residents	No. with evidence of RS virus infection—i.e., virus recovery and/or CF antibody rise
<20	28	3 (11%)	21‡ (75%)	20	20 (100%)
20–100	11	1⎫	3⎫	5	5⎫
101–400	10	2⎬ (18%)	6⎬ (30%)	5	4⎬ (79%)
401–1,280	19	4⎭	3§⎭	9	6⎭
>1,280	17	1 (6%)	3 (18%)	12	6 (50%)
Total	85	11 (13%)	36 (42%)	51	41 (80%)

* Tested with RS virus strain recovered from Junior Village outbreak using serum collected just prior to outbreak.

† Includes only individuals in residence at least 7 days during outbreak period, Dec. 4, 1966–Jan. 31, 1967.

‡ RS virus was recovered from a child in residence at least 7 days during outbreak period on Feb. 16, 1967 and this isolation is not included in the table.

§ RS virus was recovered from a child in residence as above on Feb. 2, 1967 and this isolation is not included in the table.

TABLE 6

Incidence of pneumonia and RS virus isolations according to age and vaccine status in residents of Harrison and Arthur Cottages

Age (months)	Vaccinated group			Nonvaccinated group		
	No. in group	Pneumonia No.	RS isolated No.	No. in group	Pneumonia No.	RS isolated No.
Harrison						
6–11	2	2⎱	1⎱	7†	1⎱	4†⎱
12–23	11	7⎰ (69%)*	8⎰ (69%)	40	3⎰ (9%)*	19⎰ (49%)
24–35	2	0	0	10	0	6
36–58	0	0	0	22	0	3
Total	15	9 (60%)	9 (60%)	79	4 (5%)	32 (41%)
Arthur						
6–11	0	0	0	0	0	0
12–23	2	0	1 (50%)	1	0	0
24–35	9	4 (44%)	3 (33%)	20	6 (30%)	9 (45%)
36–65	11	0	5 (45%)	9	1 (11%)	1 (11%)
Total	22	4 (18%)	9 (41%)	30	7 (23%)	10 (33%)

* Percentages significantly different ($p < .0001$)

† Includes one child 5 months of age.

The prophylactic effect of RS virus vaccination was studied by comparing the pneumonia illness rates of vaccinated and nonvaccinated individuals in residence one week or more during the outbreak (table 6). In Harrison Cottage, nine (69 per cent) of 13 vaccinees, 6–23 months of age, and only four (9 per cent) of 47 individuals in the nonvaccinated group of the same age developed pneumonia ($p < .0001$). This difference indicated not only that the vaccine was not protective but also that vaccinees experienced an altered, exaggerated clinical response to RS virus infection. (Only two of the 13 controls who were alternated with the 13 vaccinees 6–23 months of age were in residence in Harrison Cottage for at least one week during the outbreak period and neither developed pneumonia; another of the 13 controls was in residence in Arthur Cottage during the outbreak and developed pneumonia.) The vaccine did not affect significantly the susceptibility to RS virus infection as 69 per cent of vaccinees and 49 per cent of nonvaccinated Harrison Cottage children between 6–23 months of age shed the virus. The similarity of infection rates indicates that both vaccinated and nonvaccinated groups probably experienced comparable exposure to RS virus. In addition, among the Harrison Cottage residents 6–23 months of age, five of 13 vaccinees developed pneumonia serious enough to require hospitalization while none of the 47 nonvaccinated infants and children were hospitalized. One nonvaccinated Arthur Cottage resident also developed a pneumonia illness requiring hospitalization. Each of the six children hospitalized with pneumonia recovered and returned to J.V. in satisfactory condition. The 13 vaccinees who developed pneumonia received their last dose (or only dose in four instances) of RS vaccine an average of 70 days before the onset of pneumonia illness; the median was 54 days with a range of 15–236 days. The five hospitalized vaccinees received their last dose (or only

dose in one case) an average of 57 days before the onset of pneumonia illness; the median was 56 days with a range of 19–102 days.

Since 49 nonvaccinated children (26 of whom were between 6–23 months of age) and only two vaccinees were admitted to Harrison Cottage during the outbreak, the average duration of residence during the outbreak was not comparable for the two groups. Therefore, the incidence of pneumonia per child-day of residence for both vaccinated and nonvaccinated Harrison Cottage groups 6–23 months of age was calculated. Table 7 shows that after adjusting for duration of residence the rate of pneumonia per child-day in residence was more than five times greater in the vaccinated than in the nonvaccinated group ($p <$ .01) demonstrating again that the vaccinees experienced an altered, exaggerated clinical response after RS virus infection.

Table 6 also shows that an alteration of clinical response to RS vaccination was not evident among residents of Arthur Cottage where 18 per cent of vaccinees and 23 per cent of nonvaccinated children developed pneumonia. Again the RS virus isolation rate in the vaccinated and nonvaccinated groups (41 per cent vs 33 per cent) was not significantly different.

It is apparent that administration of vaccine led to an altered response to RS virus infection only among the younger nursery residents since a significant difference in illness occurred only among infants and children 6–23 months of age, almost all of whom resided in Harrison Cottage. It appeared that the alteration of clinical response was age but not antibody related as the prevalence of preoutbreak (or postvaccine) antibody among vaccinees in both cottages was comparable; each of the 9 vaccinees from Harrison and each of the 20 vaccinees in Arthur Cottage with suitable blood specimens possessed a serum antibody titer of 1:101 or greater (see table 4 for summary data

TABLE 7

Incidence of pneumonia according to child-days in residence among Harrison cottage residents 6–23 months of age

Group	Child-days in residence*		
	With onset of pneumonia	Without onset of pneumonia	Total
Vaccinated	9 (1.2%)	716	725
Nonvaccinated†	4 (0.23%)	1,724	1,728
Total	13 (0.53%)	2,440	2,453

$p <$ .01

* A child-day in residence is defined as a day in which a child is in residence at Junior Village or at D. C. General Hospital.

† Includes one infant 5 months of age.

of both cottages combined). Four of seven Harrison children 6–23 months of age with postvaccine antibody developed pneumonia; suitable postvaccine sera were not available from the other five children in this age group who developed pneumonia. Twenty-one of the 29 vaccinees in both cottages with suitable postvaccine blood specimens had antibody levels of >1:1280 and five of these vaccinees developed pneumonia, demonstrating again that high levels of RS virus serum antibody did not provide effective protection against RS virus lower respiratory tract illness.

Clinical features of illness

A summary of the clinical findings among vaccinated and nonvaccinated children with pneumonia is shown in table 8. Among the 24 children with pneumonia, the most prevalent signs were coryza, cough, and wheezing. Tachypnea and dyspnea with intercostal and in some cases substernal and/or subcostal retraction as well were observed in six of the children (five of whom had received RS virus vaccine). On physical examination each of the 24 appeared ill with notable physical findings of tympanic membrane erythema in four (17 per cent), pharyngeal

TABLE 8

Clinical findings in vaccinated and nonvaccinated pneumonia patients

Findings during illness	Vaccinated Yes No. of cases (total 13)†	Vaccinated No No. of cases (total 11)†	Maximum temperature during illness (F)	Vaccinated Yes No. of cases (total 13)	Vaccinated No No. of cases (total 11)	Duration of fever: 100.6 F or > Days	Vaccinated Yes	Vaccinated No
Coryza	13 (100%)	11 (100%)	100^6 to 101^8	1		1		1
Cough	13 (100%)	7 (64%)	102 to 102^8	1		2	3	2
Wheezing	10 (77%)	4 (36%)	103 to 103^8	8	6	3	1	2
Otitis media	3 (23%)	1 (9%)	104 to 104^8	2	4	4	2	3
Pharyngitis	6 (46%)	7 (64%)	105.0 or greater	1	1	5	2	2
Rales	13 (100%)	11 (100%)				6	4	1
X-ray evidence of pneumonitis	10* (91%)	5* (71%)				≥7	1	
Required hospitalization	5 (38%)	1 (9%)						

* X-rays taken on only 11 of 13 vaccinees and 7 of the 11 nonvaccinated children.

† 11 of the 13 vaccinees and 2 of 11 nonvaccinated children had onset of pneumonia 3 days before, day of, or day after initial RS virus isolation.

erythema in 13 (54 per cent), and fine or medium crepitant inspiratory rales in all 24. Rales occurred in patchy areas most frequently at the lung bases and occasionally throughout both lungs. Chest X-rays were taken on 18 of the children and 15 revealed findings compatible with pneumonitis. The most frequent finding on X-ray was haziness at a lung base; other occasional findings seen were infiltrations throughout both lungs, infiltrations in one or both upper lobes, bilateral interstitial infiltrations, and haziness in either a lower or upper lobe. The average maximum temperature for vaccinees was 103.4 F while that for nonvaccinated children was 103.9 F and the average duration of fever (≥100.6 F) was 4.7 days for vaccinees and 3.5 days for the nonvaccinated group. As noted previously, five of the six patients requiring hospitalization were vaccinees. At time of hospitalization, the average age of the five vaccinees was about 13 months with a range of 7–16 months, while the age of the nonvaccinated child was approximately 27 months. In general, the reasons for hospitalization were tachypnea and dyspnea with retractions. The five vaccinees, but not the nonvaccinated child, required oxygen and humidity therapy for two to five days. One of the vaccinees was placed on the critical list; however, she recovered completely. The average of the highest white blood cells (WBC) count for all six children was 15,650 cells per cu. mm. with a range of 10,200 to 23,450 per cu. mm. Differential counts for five of the six patients on either the highest or next highest WBC count ranged as follows: neutrophiles 35–80 per cent; bands 0–3 per cent; lymphocytes 15–67 per cent; basophiles 0–1 per cent; monocytes 0–2 per cent; eosinophiles 0–6 per cent (with an average of 2 per cent for the four vaccinees tested). The six children who were hospitalized with pneumonia each recovered and returned to Junior Village in satisfactory condition.

DISCUSSION

In this study, the RS vaccine not only failed to offer protection against RS virus infection and illness, but also induced an exaggerated, altered clinical response to

naturally occurring RS virus infection among the younger vaccinees. It was found that among Harrison Cottage residents 6–23 months of age, nine (69 per cent) of 13 vaccinated and only four (9 per cent) of 47 nonvaccinated individuals developed pneumonia ($p < .0001$); in addition, five of these 13 vaccinated and none of these 47 nonvaccinated children developed pneumonia serious enough to require hospitalization. Such a paradoxical effect of vaccination was not evident in Arthur Cottage which housed mostly older residents (24–65 months of age) as the pneumonia rate in the vaccinated and nonvaccinated groups in this Cottage was similar. Lack of protection against illness occurred despite the fact that the vaccine was antigenic, stimulating high levels of both neutralizing and CF antibodies. Since previous studies had shown that moderate levels of RS virus neutralizing antibody did not protect effectively against lower respiratory tract disease, we anticipated difficulty in the development of an effective RS vaccine; however, the paradoxical effect of vaccination was completely unexpected. Paradoxical vaccine effects have also been reported with rickettsial vaccines, trachoma vaccine, *Mycoplasma pneumoniae* vaccine and, more recently, inactivated measles vaccine (17–24). We have no firm explanation for the paradoxical effect of RS virus vaccination which in some way induced an alteration of host response to natural RS infection.

A previously reported hospital study showed that the highest rate of RS virus associated lower respiratory tract disease occurred in the first four months of life when passively transferred maternal antibody was at high levels (5). The failure of maternal antibody to protect against RS virus infection, plus the demonstration in the present study that vaccinees experienced an altered, exaggerated clinical response after natural RS virus challenge, suggest that serum antibody may play an active role in the pathogenesis of RS

virus disease. This interpretation of the role of serum antibody does not deny the pathogenic potential of RS virus which is capable of producing pulmonary involvement in the absence of detectable humoral antibody (see table 5).

The possibility that "local" antibody present in secretions of the respiratory tract would be more effective than serum antibody as a defense mechanism against certain illnesses in which the portal of entry of the etiologic agent was not only superficial but also constituted the area of primary injury was suggested by Francis about 25 years ago (25–27). In a recent adult volunteer study it was observed that antibody in nasal secretions had a more important role in resistance to experimentally induced parainfluenza virus type 1 reinfection than did serum antibody (28). If a similar situation exists in RS virus infection, this might explain why enhancement of RS virus illness did not occur in the older vaccinees, 24–65 months of age, who presumably had had more prior experience with RS virus infection than did the younger vaccinees (as determined by comparing the preoutbreak neutralizing antibody titers of nonvaccinated residents 24–65 months of age with those of the nonvaccinated residents 6–23 months of age in table 4). As a result of such previous infection, it is possible that local respiratory tract antibody was induced and that this antibody acted to inhibit or modify RS virus infection in the older vaccinees. If these suppositions are correct, then both the increased incidence of RS virus lower respiratory tract illness in infants with passively acquired maternal antibody and the enhancement of RS virus illness among the younger vaccinees may have resulted from the interaction of serum antibody and RS antigen in the lungs of individuals who lacked respiratory tract secretory antibody or who possessed a low level of such antibody. By stimulating serum antibody in individuals without or with low levels of local antibody the in-

activated vaccine may have created arti-
ficially an internal environment similar to
that seen in infants below six months of
age, i.e., high serum levels and low or no
local respiratory tract antibodies. Al-
though in the hospital study mentioned
above, an age factor, rather than interac-
tion of serum antibody and RS virus, might
account for the high rate of lower respira-
tory tract illness in infants one to four
months of age, our Junior Village study
showed that age alone was not the pre-
disposing factor to altered reactivity to
RS virus infection. In our study, this altered
response was limited to the vaccinated
group, although both vaccinated and non-
vaccinated groups 6–23 months of age were
apparently effectively exposed to RS virus.
It should be emphasized that nasal wash-
ings were not available for testing for RS
virus antibody in this J.V. study and that
epidemiologic information defining the role,
if any, of local RS virus antibody does not
exist. Studies evaluating the role of local
respiratory tract antibody in RS virus in-
fection are urgently needed to help answer
the perplexing questions raised by the un-
expected findings of this study.

The possibility that delayed hyper-
sensitivity played a role in the paradoxi-
cal effect of vaccination cannot be ruled
out. However, it is not probable that such
a mechanism could be involved in the
pathogenesis of naturally occurring lower
respiratory tract disease of early infancy
since cell mediated delayed hypersensitiv-
ity is not transferred from mother to in-
fant. It is unlikely that monkey kidney
antigens, either in sedimentable form un-
associated with virus or incorporated into
the virion envelope, were responsible for
the exaggerated clinical response. Pre-
liminary evidence reported by Parrott et
al. would tend to support this view; it was
found that during a study of two vac-
cines, one an RS virus vaccine identical to
that used in the J.V. study and the other
a parainfluenza virus type 1 vaccine pre-
pared in a manner almost identical to that

of the RS vaccine, four of five RS virus
vaccinees and none of five parainfluenza
virus type 1 vaccinees infected with RS
virus required hospitalization (7).

From the observations made in this
study we would recommend that inacti-
vated RS vaccines, regardless of anti-
genicity, not be given parenterally unless
definitive information emerges concerning
the mechanism of potentiation of RS virus
infection in infants vaccinated with inac-
tivated RS vaccine and this information in-
dicates that a new and different approach
to parenteral immunoprophylaxis could be
safely and confidently undertaken. In ad-
dition, since the mechanism of potentiation
is not known, studies investigating the
efficacy of RS vaccines administered by
other routes should be approached with
caution; administration of gammaglobulin
to infants and children during periods of
RS virus prevalence should also be ap-
proached with caution since the passive
transfer of RS virus antibody via gamma-
globulin administration might induce an
alteration of host response to RS virus in-
fection.

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