RECOVERY FROM INFANTS WITH RESPIRATORY ILLNESS OF A VIRUS RELATED TO CHIMPANZEE CORYZA AGENT (CCA)

II. EPIDEMIOLOGIC ASPECTS OF INFECTION IN INFANTS AND YOUNG CHILDREN [1]

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Introduction

Morris, Blount and Savage (1) studied an outbreak of coryza in a colony of chimpanzees held under observation for 3 to 24 weeks prior to the onset of illness. A virus (CCA) was recovered from one of the 14 affected chimpanzees and the remaining 13 animals developed antibody to this virus during convalescence. A person working with the infected chimpanzees subsequently experienced a respiratory infection and, although virus isolation attempts were unsuccessful, a rise in antibody for CCA virus was observed during convalescence. When susceptible chimpanzees were inoculated intranasally with tissue-culture CCA virus, coryza was observed after a 3-day incubation period. These findings suggested the possibility that CCA was a virus of human origin which produced an outbreak of mild respiratory illness when introduced into a susceptible population of chimpanzees.

During a study of infants with lower respiratory disease (2) two agents (Long and Snyder) were recovered which were indistinguishable from CCA virus. The infants from whom the viruses were recovered developed antibody during convalescence. From these findings it is clear that viruses which are indistinguishable from CCA virus are capable of infecting human beings. These results cannot be interpreted to mean that these agents were the cause of the illnesses in the infants, since the temporal association of disease and infection with an agent is only the first step in the chain of evidence required for etiologic significance (3).

This communication will describe preliminary investigations which were designed to provide an understanding of the epidemiology and pathogenicity for infants of the CCA-Long-Snyder group of agents. Infants with severe lower respiratory illness and a control group of infants without such illness were studied. Mild respiratory illnesses were not studied because of the frequency with which these illnesses occur during the winter months in this age group (4), and since clinical diagnosis is less

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accurate than in infants with severe illness.

Materials and methods

Study population. The study population consisted of infants and children under 4 years of age with severe lower respiratory illness and a control group of infants and small children who did not have respiratory illness at the time specimens for virus isolation were obtained. The subjects were from a low socioeconomic environment.

Infants and small children up to the age of 4 years who were admitted to either the Harriet Lane Home of the Johns Hopkins Hospital, Baltimore, Md., or the Pediatric Division of the Baltimore City Hospitals with the diagnosis of bronchopneumonia or bronchiolitis constituted the hospital respiratory group. Patients up to 4 years of age with bronchopneumonia who were seen in the outpatient clinic of the same institutions constituted the outpatient respiratory group. Bronchopneumonia was diagnosed when tachypnea, fine moist râles and, in most instances, pulmonary infiltration occurred. The clinical course of the patients selected for this study did not resemble that of a bacterial pneumonia. Chest x-rays were taken on all ward patients with pneumonia and on the majority of the outpatients with pneumonia. Bronchiolitis was diagnosed when tachypnea, expiratory wheezing, prolongation of expiration, and evidence of emphysema were observed. In addition, it was not uncommon for moist râles and x-ray evidence of pulmonary infiltration to occur in infants with bronchiolitis.

The controls were children with nonrespiratory illnesses from the outpatient clinics of the Harriet Lane Home and Baltimore City Hospitals and from the pediatric ward of the latter hospital. Patients with infectious disease were not admitted to this ward.

The study extended from October, 1956, to March, 1957. The respiratory patients and the controls were distributed over this 6-month interval.

The same procedure was employed in

Table 1

Composition of respiratory and control groups

	Lower respiratory illness									Controls					
	Pneumonia						Bronchiolitis								
	OPD			Hospital			Hospital			OPD			Hospital		
	H.L.H.*	B.C.†	Total	H.L.H.	B.C.	Total	H.L.H.	B.C.	Total	H.L.H.	B.C.	Total	H.L.H.	B.C.	Total
Virus isolation attempts plus serologic study of paired sera	6	7	13	13	13	26	46	28	74	8	95	103	0	29	29
Virus isolation attempts only	0	0	0	1	1	2	9	7	16	0	17	17	0	2	2
Average age (months)‡	16	19	18	9	9	9	7	4	6	21	22	22	0	19	19
Average interval between serum samsamples (weeks)	7	7	7	6	5	5	6	7	6	6	6	6		8	8

* Harriet Lane Home.
† Baltimore City Hospitals.
‡ Average age of group from whom both throat swabs and paired sera were available.

TABLE 2

Antibody response to Long virus as detected by the complement-fixation and neutralization techniques

Group	CF antibody rise*			Neutralizing antibody rise†		
	Tested	Positive		Tested	Positive	
		Number	Per cent		Number	Per cent
Respiratory	121	22	18.1	73	32	43.8
Control	132	10	7.6	70	8	11.4
Totals	253	32	12.6	143	40	27.9

* Four-fold or greater.

† Five-fold or greater. In all but one instance the rise was 8-fold or greater.

studying the various groups. A throat swab and a blood specimen were obtained at the time of admission to the hospital or during the first clinic visit. An attempt was made to obtain a second serum sample 4 weeks later. In many instances a second sample could not be secured until after an interval of 6 to 10 weeks, and in several instances 12 weeks.

Table 1 shows the number of children in each group, their average age in months and the mean intervals between serum samples. It will be seen that the controls differed from the respiratory illness cases in that the proportion of hospitalized individuals was smaller. The controls were, on the average, older than the patients with respiratory illness.

Laboratory procedures. The techniques for virus isolation, complement-fixation (CF) and neutralization tests in tissue culture have been described (2).

RESULTS

Comparison of neutralizing and CF antibody response to infection with Long virus. The results of testing paired serum specimens for a rise in CF and neutralizing antibody to Long virus are presented in table 2. All of the subjects were tested by CF, whereas only 58 per cent of these individuals were tested by the neutralization technique. In the total group, proportionately two times as many antibody rises were detected by the neutralization technique as by CF. The lowest serum dilution tested by CF was 1 in 8. If a dilution of 1 in 2 or 1 in 4 had been employed, it is possible that the results of the CF test would more closely approximate those of the neutralization method.

The antibody response of individuals tested by both the neutralization method and CF is shown in table 3. The proportion of persons tested by the neutralization technique in the group who developed CF antibody (20 to 32 (62 per cent)) was slightly greater than in the group who failed to develop this antibody (123 of 213 (58 per cent)). Thus a slight bias was present in favor of individuals who developed CF antibody. Nevertheless, 89 per cent of the 45 individuals with an antibody rise of any type developed neutralizing antibody, whereas only 44 per cent developed CF antibody. Thus, in infants and small children the neutralization technique was at least twice as sensitive as CF in de-

TABLE 3

Antibody response to Long virus in individuals tested by both CF and neutralization techniques

Category	Respiratory group		Controls		Total	
	No.	Per cent	No.	Per cent	No.	Per cent
Tested	73	100	70	100	143	100
No antibody rise	38	52	60	86	98	69
Antibody rise*						
CF and neutralization	13	18	2	3	15	10
CF only	3	4	2	3	5	3
Neutralization only†	19	26	6	9	25	17
Total by either method	35	48	10	14	45	31
Total CF	16	22	4	6	20	14
Total neutralization	32	44	8	11	40	28

* Four-fold or greater rise of CF antibody. Five-fold or greater rise of neutralising antibody; in all but one instance the rise of neutralizing antibody was 8-fold or greater.

† CF antibody not detected at a dilution of 1 in 8 in the convalescent serum.

tecting serologic evidence of infection with Long virus.

An increase in neutralizing antibody was observed for all but 5 of the 20 individuals who developed CF antibody. Of these 5, three showed a high level of neutralizing antibody in both sera while the remaining two persons failed to develop detectable neutralizing antibody. In the former group it is possible that the first serum specimen was obtained at a time after neutralizing antibody had risen to high levels but before the appearance of CF antibody.

A possible effect of age on the CF antibody response to Long virus was observed. In the group of 45 persons with an antibody response shown in table 3, 8 (62 per cent) of 13 individuals over 1 year developed CF antibody, whereas a rise in this antibody was observed for only 12 (37 per cent) of 32 infants less than 1 year of age. The difference is not statistically significant but is suggestive.

Occurrence of neutralizing antibody for Long virus in control children of various age groups. As shown in table 4, 55 per cent of control infants in the first half year of life possessed neutralizing activity for Long virus in their serum. During the second half of the first year the proportion of infants with neutralizing activity decreased to 27 per cent, suggesting that

TABLE 4

Neutralizing antibody for Long virus in the serum of control infants and children, by age

Age (*months*)	Tested	Serum neutralization titers of 1 in 8 or greater*	
		Number	Per cent
1–5	18	10	55
6–11	15	4	27
12–23	23	11	48
24–35	13	10	77
36–47	10	8	80

* Only the first of the paired serum specimens included in this tabulation.

the high frequency of positive reactors during the first 6 months represents, in part, the effect of transplacentally acquired maternal antibody. In the second, third and fourth years of life the proportion of individuals with neutralizing activity in their serum increased, suggesting that Long virus had been active in the community during the past 4 years. The large proportion of 3- and 4-year olds with serum neutralizing activity further suggests that initial infection with Long virus is an uncommon event in our study population after the age of 4 years.

The hospital as a source of infection with Long virus. The incidence of serologic evidence of infection with Long virus in the clinic and ward control groups is shown in table 5. When the incidence of development of CF antibody or neutralizing antibody without CF antibody or either type of antibody was compared for the two groups, a significant difference was observed. These findings indicate that infection with Long virus occurred 5 to 8 times more frequently among control infants who were hospitalized than among outpatient controls. Although the longer mean interval between serum specimens for the ward group (table 1) may have contributed in small part to the observed difference, it is probable that a higher rate of infection on the ward was responsible for these findings.

Association of the ward controls with patients with known severe respiratory illness could not be implicated, since the former infants were admitted to a separate ward from which patients with infectious disease were excluded. Lower respiratory illness (pneumonia, bronchiolitis or croup) was not observed in the ward control group, although a history of mild upper respiratory illness occurring between the time of the first and second serum specimens was commonly obtained. In the clinic control group mild upper respiratory infections commonly occurred between the time of the first and second serum collection. In this connection the findings of Badger et al. (4) that young children suffer an average of 8 recognizable upper respiratory infections per year may be mentioned.

Infection with Long virus occurred throughout the 5 months the ward controls were under observation and during 4 of the 6 months the clinic control group was under study.

Relation of Long virus to pneumonia and bronchiolitis of infancy. Long virus was isolated once in KB tissue culture from the throat swab fluid of a clinic infant with bronchopneumonia. Twelve other isolation attempts with throat

TABLE 5

Influence of residence on hospital ward upon acquisition of antibody to Long virus in control group

Group	Number with rise in CF antibody during indicated month								Number with rise in neutralising antibody without a rise in CF antibody during indicated month								Per cent with antibody rise by either technique
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total		
							No.	Per cent							No.	Per cent	
Clinic	1/34	1/16	0/11	0/18	0/11	1/13	3/103	3*	0/13	1/8	1/7	0/11	0/7	0/5	2/51	3.9	6.9
Ward	0/7	4/12	1/4	2/4	0/2		7/29	24*	1/4	1/6	0/3	1/4	1/3		4/19	21.0	45.

* When tested for significance $\chi^2 = 10.7$ and $P = 0.001$.

TABLE 6

Rise in CF antibody to Long virus in infants hospitalized for bronchopneumonia, bronchiolitis or nonrespiratory illnesses

Hospital	Control group		Pneumonia			Bronchiolitis		
	Tested	Positive (rise)	Tested	Observed positive	Expected positive*	Tested	Observed positive	Expected positive*
Harriet Lane			13	3	3.1	46	5	11
Baltimore City	29	7	13	4	3.1	28	5	6.7
Totals	29	7	26	7	6.2	74	10	17.7

* Number expected to show rise if rate in control group had prevailed.

swabs from clinic infants and children with bronchopneumonia were unsuccessful as were isolation attempts with specimens from 28 infants with pneumonia and 90 infants with bronchiolitis who were hospitalized. Long virus was not isolated from the throat swabs of 151 control individuals. The throat swabs of respiratory patients and controls who developed a rise in antibody to Long virus were tested in liver epithelium cultures with negative results except for the one patient from whom virus was isolated in KB culture.

In the group of infants hospitalized with pneumonia or bronchiolitis the incidence of infection with Long virus as determined by CF (table 6) or the neutralization technique was not significantly different from that of the control group. The control group was not completely satisfactory in that the average age was considerably older than the average for either respiratory group (table 1). However, the control infants whose age matched that of the respiratory patients exhibited the same incidence of CF antibody development (3 of 12 (25 per cent)) as the older controls (4 of 17 (24 per cent)). It is possible that some of the respiratory illnesses which required hospitalization were associated with Long virus but this would be difficult to establish because of the high rate of infection in the control group.

The incidence of Long virus infection among outpatients with bronchopneumonia and among outpatient controls is shown in table 7. The mean age and mean interval between serum samples were similar in both groups (table 1).

TABLE 7

Incidence of Long virus infection among clinic infants with pneumonia and clinic infants with nonrespiratory illness

Test procedure	Control group		Pneumonia		
	Tested	Positive (rise)	Tested	Observed positive	Expected number positive*
Complement fixation	103	3	13	5	.4
Complement fixation plus neutralization	51	4	13	9	1

* Number expected to show rise if rate in control group had prevailed.

It is important to note that the 13 individuals with bronchopneumonia were distributed over the 6-month period of the study and that cases of bronchopneumonia with an antibody rise for Long virus occurred during each of the months except December. The significantly higher rate of infection among

TABLE 8

Antibody response to Long and adenoviruses of 13 outpatients with bronchopneumonia

Patient	Age (*months*)	Interval between serum specimens (*weeks*)	Serum	Reciprocal of antibody titer with indicated virus			Bacterial pathogens in nose and throat cultures
				Long		Adeno. CF*	
				CF	Neut.		
Long	17	8	Acute	<4	<4	8	
			Conval.	80	64	16	0
Fal.	24	6	Acute	<4	<4		
			Conval.	64	64	<8	0
Ful.	36	4	Acute	<4	8		
			Conval.	16	64	<8	N.D.†
Sl.	17	7	Acute	<4	<4		Pneumo-
			Conval.	32	64	<8	coccus
Ha.	36	5	Acute	4	128		
			Conval.	16	64	<8	N.D.
Wa.	7	11	Acute		<4	4	
			Conval.	<8	80	8	0
Pe.	8	8	Acute		<4		
			Conval.	<8	16	<8	0
Sw.	15	6	Acute		<4		β hemo.
			Conval.	<8	40	<8	strep.
Bu.	6	5	Acute		<4	<4	
			Conval.	<8	64	16	0
Ga.	24	10	Acute		<4	<4	
			Conval.	<8	<8	8	0
Sta.	20	4	Acute	256	128		
			Conval.	64	256	<8	0
Le.	6	7	Acute		8	<4	
			Conval.	<8	20	64	N.D.
Je.	13	8	Acute		<4		Pneumo-
			Conval.	<8	<8	<8	coccus

* Type 2 adenovirus.

† N.D. = not done.

the outpatients with bronchopneumonia suggests that an etiologic association exists between Long virus and a certain segment of the bronchopneumonia syndrome. However, since the number of outpatients with bronchopneumonia was small (13 individuals), any conclusions derived from this study must be regarded as tentative in nature. The firm establishment of an etiologic association between Long virus and bronchopneumonia must await future studies.

Detailed information on the 13 clinic patients with bronchopneumonia is given in table 8. The isolation of pneumococcus or β hemolytic streptococcus from 3 of the 10 nose and throat cultures does not constitute an unusually high proportion of positive findings during the winter months as judged by the results presented by Rabe (5). The clinical course of the 3 patients from whom bacterial pathogens were recovered was similar to that of the other patients with pneumonia and did not resemble a bacterial illness.

Discussion

The isolation from infants with lower respiratory disease of two viruses which were indistinguishable from CCA virus indicated that agents of this group are capable of infecting human beings (2). Additional support for this conclusion was the finding that 41 infants with lower respiratory illness and 16 control infants without respiratory disease developed CF antibody, neutralizing antibody, or both, to Long virus during the course of this study.

The frequent occurrence of infection when compared with the infrequent isolation of virus suggests that Long virus is recoverable from the throat during a very short interval following infection. However, it is probable that many of the infections diagnosed by serologic methods in this study occurred after the throat swab was obtained and during the interval between the collection of serum samples. Morris, Blount and Savage (1) experienced similar difficulty in isolating CCA virus from chimpanzees with respiratory illness. In the epizootic of coryza studied by these investigators virus was recovered from only one of 14 affected chimpanzees 4 days after the first respiratory signs were noted.

Acquisition of neutralizing antibody to Long virus occurred very early in life and at a rapid rate. In the present study population, 77 per cent of children 3 years of age and 80 per cent of children 4 years of age possessed neutralizing activity in their serum. From these findings one would anticipate that infection of older individuals would occur infrequently. However, Morris, Blount and Savage (1) reported that 24 per cent of individuals in the age group 10 to 18 years possessed CF antibody for CCA virus. We have observed a similar incidence of CF antibody for Long virus in a group of 67 prisoners (21 through 29 years old) from a Federal prison in Ohio.

Assuming that CF antibody is a measure of recent or persisting infection the findings just described could result from: (*a*) the occurrence of reinfection during the second and third decade at a time when neutralizing antibody levels have decreased below a certain critical level, (*b*) the occurrence of persisting infection in a certain proportion of individuals, (*c*) differences in the make-up of the various study populations, or (*d*) the occurrence of antigenically related but distinct agents which share a common CF antigen with Long virus. At the present time it is not possible to

determine which of these alternatives offers the correct explanation.

The available data suggest that the fourth possibility is unlikely and that Long virus belongs to a group with only one antigenic type. If this virus belonged to a group whose members shared a common CF antigen but reacted in a more specific manner in the neutralization reaction, such as the adenovirus group (6), one would expect to encounter more individuals who developed CF antibody than neutralizing antibody for Long virus. The opposite situation occurred in this study. Fifty-six per cent of the infants and children with an immune response developed neutralizing antibody without a detectable CF response. In addition, a rise in neutralizing antibody accompanied the development of CF antibody in the majority of instances (75 per cent). However, since certain individuals developed CF antibody without a neutralizing antibody rise the possibility that Long virus belongs to a group with multiple antigenic types can not be disregarded.

The hospital as a source of infection with Coxsackie viruses was studied by Parrott, Huebner, McCullough, Wolf and Naiden (7). Their investigation indicated that hospitalized children became infected with Coxsackie A viruses while on the ward. Viruses were isolated from patients who had been in the hospital for 10 days or more and from whom two or more negative anal swabs had been previously obtained. In the present study, additional evidence was obtained that the hospital can serve as a center for virus dissemination. Thus, the rate of infection with Long virus among infants and children who were hospitalized was 6 times greater than in the clinic population. Approximately one of every two individuals without respiratory illness who were admitted to the ward developed antibody to Long virus. This is in contrast to a rate of antibody development of 7 per cent in the clinic control group.

An association between Long virus and lower respiratory disease in hospitalized infants was not demonstrable. Possibly, however, such an association does exist but was overshadowed by the high rate of infection among the hospital control group. The lower rate of infection among the clinic controls was considerably less than that for outpatients with bronchopneumonia. Although this observed difference appears to be significant, the number of clinic bronchopneumonia patients was sufficiently small (13 individuals) that the conclusions derived from the data must be considered as tentative.

At the present time a definite statement cannot be made regarding the spectrum of pathogenicity of Long virus for man. It is possible to state that this virus was prevalent among young children in this community during the study period and that in the majority of infants infection resulted in either mild illness or no discernible disease.

Since (*a*) CCA virus has been shown to produce mild upper respiratory illness in chimpanzees, (*b*) Long and Snyder viruses were recovered from the throat of infants with lower respiratory illness, (*c*) Long and Snyder viruses are indistinguishable from CCA virus, (*d*) Long virus is suspected of an association with bronchopneumonia in infancy, and (*e*) the striking characteristic of these viruses is the production of syncytial areas in tissue culture, it is suggested that these agents be grouped together and named "respiratory syncytial" (RS) virus until their epidemiology and pathogenicity are better understood.

Summary

A study was carried out to elucidate the epidemiology of an agent (Long virus) which was recovered from an infant with bronchopneumonia and shown to be indistinguishable from chimpanzee coryza agent (CCA). The neutralization test was found to be twice as sensitive as the complement-fixation technique in the serologic diagnosis of infection in infants and small children. In the present study population, 80 per cent of children 4 years of age possessed neutralizing activity for Long virus in their serum. Seven per cent of nonhospitalized control infants and children, without overt respiratory illness at the time of initial bleeding, developed antibody for Long virus during an average interval of 6 weeks in the winter months. The rate of infection among a similar control group who were hospitalized on a ward which did not admit patients with infectious disease was 6 times greater than among the outpatient controls from the same hospital.

An association of Long virus with severe lower respiratory illness in hospitalized patients could not be demonstrated, possibly because of the high rate of infection occurring in the ward control group. The data suggested an association between Long virus and bronchopneumonia occurring in outpatients. However, the number of outpatients with bronchopneumonia was small (13 individuals) and thus the conclusions derived from these data must be considered tentative.

It is proposed that the agents isolated in this study and the CCA virus be grouped together and named "respiratory syncytial" (RS) virus.

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