Respiratory Syncytial Virus Neutralizing Activity in Nasal Secretions Following Natural Infection* (33946)

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(Introduced by J. C. Houck)

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Recent studies in volunteers have demonstrated that the presence of local secretory IgA antibody against parainfluenza type 1 virus is more important than serum antibody in protection against subsequent homotypic virus infections (1, 2). It seems reasonable that this would be the case with other paramyxoviruses whose site of entry, replication and pathologic expression is limited similarly to the respiratory tract. Because of the importance of respiratory syncytial (RS) virus infection in infants and children, the nasal secretory antibody response was studied in 17 infants and children whose illness was due to naturally occurring RS virus infection. The findings, presented here, have implications for immune prophylaxis and for understanding the pathogenesis of RS virus illness in infants and children.

Materials and Methods: Serum and nasal secretions were obtained from infants and children in whom there was reasonable clinical suspicion that their illness might have been due to RS virus infection. Specimens were obtained at the time of admission to the hospital and approximately 3 weeks thereafter. Findings are reported only for those in whom RS virus infection was proven by virus recovery and/or a fourfold or greater rise in serum complement fixing (CF) antibody following illness. The nasal secretions were collected by gentle suction from the nostril following the periodic instillation of small amounts of 0.15 M saline solution. None of the secretions tested was grossly contaminated with blood. Secretions were stored at −20° prior to being processed and tested for neutralizing antibody.

After dialysis against 0.015 *M* saline, secretions were concentrated by lyophilization, reconstituted to one-tenth of the original volume and tested for levels of IgA, IgG, and IgM by the radial immunodiffusion technique using immunoplates (Hyland Laboratories) (2). In addition, each of the specimens was assayed on plates prepared in our laboratory with specific anti-immunoglobulin sera kindly supplied by Dr. John L. Fahey, National Cancer Institute. The results obtained with both types of plates agreed within a precision of 10%. Varying dilutions of serum obtained from a single donor were used as standards in all tests.

Secretion concentrates were adjusted to IgA levels of approximately 10–25 mg/100 ml. The IgG and IgM levels measured were all in the range expected for nasal secretions containing the observed levels of IgA; thus, the IgG and IgM which were present did not appear to result from the extravasation of blood into the secretions. Neutralizing activity in the reconstituted and adjusted secretions was measured by the RS virus plaque reduction technique described below. Because of variation in the amount of IgA in different specimens, even after processing, the neutralizing activity levels are presented in Table I as actually determined and as adjusted for an IgA level of 10 mg/100 ml.

Complement fixation antibody in serum was assayed by a micromethod using 1.7 units of complement, 16 units of RS virus antigen and overnight incubation at 4° (3, 4).

Nasal secretion neutralizing activity was measured by a plaque reduction technique using the Long strain of RS virus (5). An RS

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TABLE I. Neutralizing Activity in Nasal Secretions of Infants and Children with RS Virus Lower Respiratory Tract Disease.[a]

Patient no.	Age (months)	Virus recovery	Reciprocal of serum RS CF antibody (acute/conval.)	Nasal secretions		
				IGA (mg/100 ml; acute/conval.)	Reciprocal of RS plaque reduction titer	Reciprocal of RS plaque reduction titer adjusted to 10 mg/100 ml of IGA
1	11	+	<4/64	17/11	<4/225	1/205
2	17	+	<4/32	12/23	<4/1000	2/435
3	12	0	4/64	14/22	80/450	57/205
4	40	0	4/64	33/16	4/35	1/22
5	6	+	<4/32	13/14	5/110	4/79
6	4	+	4/16	18/11	7/20	4/18
7	33	0	<4/8	16/26	10/130	6/50
8	4	+	<8/<8	35/13	130/80	37/62
9	2	+	<8/<8	24/14	300/270	125/193
10	11	0	<4/8	20/15	40/60	20/40
11	35	0	<4/64	19/14	80/30	42/21
12	21	0	<4/64	35/26	400/100	114/38
13	15	0	256/1024	16/15.5	>1000/80	625/52
14	10	0	<4/16	13.5/15	6/2	4/1
15	19	0	<4/64	17/18	7/14	4/8
16	4	+	<4/8	15/16	24/5	16/3
17	15	0	<4/32	24/14	60/20	25/14

[a] Note: <1:4 is considered as 1:2 for adjusting RS plaque reduction titer to 10 mg/100 ml of IGA.

virus suspension prepared in Eagle's medium with 10% fresh guinea pig serum was incubated with an equal volume of diluted, inactivated (56° for 30 min) nasal secretion for 1 hr at room temperature; the concentration of RS virus in the suspension was calculated to yield 20–50 plaques/60-mm petri-dish culture of HEp-2 cells. Then, 0.2 ml of each virus–secretion mixture was inoculated onto 3 HEp-2 plate cultures which were incubated at 37° for 2 hr in a humidified atmosphere containing 5% CO_2. After one washing with Hanks' balanced salt solution, 3 ml of Eagle's medium number 2 containing 10% heat-inactivated fetal calf serum and 0.75% methyl cellulose were added to each culture plate (5). The HEp-2 cultures were then incubated in a humidified atmosphere of 5% CO_2 at 37° for 3–4 days, fixed with formalin, and stained with hematoxylin and eosin. Plaques were counted with a dissecting microscope. The titer of neutralizing activity which produced 60% reduction of RS virus plaques was calculated by graphing on probit paper the percentage of plaque reduction produced by the various nasal secretion dilutions (5).

Throat and anal swab specimens from study patients were inoculated into HEp-2, primary rhesus monkey kidney cell and human embryonic kidney cultures. Supernatant fluids from cultures which showed a cytopathic effect (CPE) suggestive of RS virus infection were passaged and the resulting cultures which exhibited CPE were tested for presence of RS antigen by CF using specific RS ferret antiserum (5).

Results. The patients included in the present study were admitted to the hospital between November 1966 and March 1967, the majority between December and March, when RS virus was prevalent in the community and when there was a dramatic upsurge in hospital admissions for lower respiratory tract disease in infants and young children. The infants and children in this study were between 2 and 40 months of age. In most instances the clinical diagnosis was bronchiol-

itis or bronchopneumonia.

During the study interval, a total of 100 pediatric patients was admitted to the hospital with a lower respiratory tract illness associated with RS virus infection. However, paired acute and convalescent phase nasal secretions containing sufficient IgA for the determination of RS virus neutralizing activity were available from only 17 patients. As shown in Table I, RS virus infection was detected in 7 of these individuals by recovery of virus and in 15 by a rise in serum CF antibody during convalescence.

In 7 of the 17 patients, an approximately fourfold or greater rise in nasal secretory neutralizing activity (adjusted to 10 mg/100 ml of IgA) developed during convalescence. Three of the patients exhibited a fourfold or greater decline in secretory neutralizing activity during convalescence. The quantity of nasal secretions available following processing, adjustment to 10–35/100 ml of IgA and testing for RS neutralizing activity was not adequate to permit identification of the immunoglobulins responsible for such neutralizing activity.

Nine of the 17 patients possessed neutralizing activity in acute phase secretions at a level of 1:16 or greater (adjusted to 10 mg/100 ml of IgA). The presence of nasal secretory neutralizing activity at this level appeared to bear a relationship to the likelihood of a subsequent response; only one of 7 patients who developed a rise in neutralizing activity had an initial titer of 1:16 or greater, whereas this level of activity was found in the acute phase secretions of 8 of 10 individuals in whom a response did not develop. The recovery of RS virus also may be related to the development of a rise of secretory neutralizing activity; such a response developed in 4 of the 7 patients from whom virus was recovered but in only 3 of 10 subjects who did not yield virus.

Age did not seem to be related to the development of neutralizing activity in nasal secretions following RS virus disease nor did age seem to be related to the presence of activity at a titer of 1:16 or greater during the acute phase of illness.

Discussion. RS virus infection is the single most important cause of respiratory tract disease in infants and children. A major share of the resultant morbidity and mortality occurs early in life at a time when the level of maternally derived serum antibody is moderately high (6, 7). This has led us to postulate that serum antibody, in the presumed absence of local antibody, is important in the pathogenesis of RS virus disease (7). This hypothesis is supported by the finding that administration of an inactivated RS virus vaccine which stimulated the development of high levels of serum antibody did not prevent infection (8, 9). In addition, when infants who received this vaccine underwent natural infection with RS virus they had illnesses which were more serious than those which occurred in a concurrent control group. Furthermore, some of the vacinees had bronchiolitis at an age which was considerably beyond that characteristic of the unmodified naturally occurring disease.

These observations clearly demonstrate that RS serum neutralizing antibody did not protect against infection or illness. As a result we have turned our attention to the potential role of respiratory tract secretory antibody since it has been shown to be the major determinant of resistance to infection with another paramyxovirus, type 1 parainfluenza virus (1, 10). An understanding of the natural history of nasal secretory antibody in RS virus infection will undoubtedly clarify certain aspects of the pathogenesis of RS illness and provide a sounder basis for its prevention.

The findings from the current study represent the first demonstration of the development of neutralizing activity, presumably antibody, in nasal secretions during acute respiratory syncytial virus infection. Since this information came from cross-sectional studies of acute illness, it was not possible to describe completely the sequence of development of neutralizing activity or to determine its full significance.

A significant proportion of infants and children had moderately high levels of neutralizing activity in their secretions when admitted to the hospital. This unexpected finding could be a manifestation of (i) rapid development of nasal secretory antibody, (ii) prior infection with RS virus, or (iii) the

presence of nonspecific virus inhibitor in nasal secretions. Rapid development of nasal antibody can be proven only after longitudinal study of antibody in secretions collected prior to exposure to RS virus and frequently thereafter. However, the occurrence of elevated levels at the time of acute illness, even in infants, and the fact that there were four instances of a threefold or greater fall in antibody following illness suggests that nasal antibody response occurs early in infection and also rapidly declines. Since the time of sampling of patients in our study was determined by the time of their hospitalization, it is probable that some subjects were studied relatively early and others relatively late in the course of their infection.

Prior infection seems unlikely as the full explanation for moderately high levels of acute phase secretory antibody since the distribution of elevated initial levels was similar in infants 2–6 months of age and in older individuals. The former were not born until after the prior community-wide RS virus outbreak. Nevertheless, we cannot rule out the possibility that some of the initial elevated levels represent residual antibody from prior infection or represent accelerated response conditioned by prior infection. Whatever the source of acute nasal secretion neutralizing activity, its presence may influence the detection of RS virus in oropharyngeal secretions when certain patients are sampled on admission to the hospital.

A nonspecific virus inhibitor could be responsible for the neutralizing activity detected in acute phase secretions, especially those of the young infants. However, there is no other evidence for the existence of nonspecific RS virus inhibitors in nasal secretions. The normal distribution of titers of RS virus neutralizing activity in the nasal secretions of 100 young adults, as determined by Mills, suggests the RS plaque-technique measures specific neutralizing antibody rather than nonspecific inhibitor (11).

Virtually no information is available regarding the development of competence of the respiratory tract secretory IgA system in early infancy. The present studies suggest that the system does operate quite early in life and at a reasonable level of efficiency. In terms of immune prophylaxis in early infancy, therefore, these studies suggest that it might be reasonable to stimulate this system to induce protective immunity.

Summary. The development of RS virus neutralizing activity in nasal secretions was documented in 7 of 17 infants and children who were hospitalized for RS virus lower respiratory tract disease. Most of the patients in whom a rise in secretory neutralizing activity did not occur possessed moderately high levels of such activity in secretions collected at the time of admission to the hospital. At present we favor the view that this acute phase neutralizing activity represents antibody which developed early in the course of infection (possibly earlier than serum antibody) rather than the alternate possibility of nonspecific inhibitors in secretions.

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