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Secretory Immunological Response in Infants and Children to Parainfluenza Virus Types 1 and 2

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The secretory immunological responses to natural infection with parainfluenza viruses are not well defined. Nasopharyngeal secretion specimens from 20 infants and children naturally infected with parainfluenza virus type 1 or type 2 were examined for class-specific antibody and virus-neutralizing activity. There was a marked discordance in individual secretions between immunoglobulin A (IgA) antibody (as measured by indirect immunofluorescence) and neutralizing activity (as determined by either hemadsorption plaque or 50% tissue culture infective dose reduction) to the infecting parainfluenza virus type. Many secretions contained neutralizing activity in the absence of detectable IgA antibody; conversely, secretions with measurable IgA antibody frequently lacked neutralizing activity. Moreover, there was no relationship between neutralizing activity and the course of illness. All 11 patients with serial secretion specimens showed a fourfold or greater titer rise in IgA antibody to the homologous parainfluenza virus type. Antibody usually appeared 7 to 10 days after the onset of symptoms and peaked at about 2 weeks. This response did not appear to be related to age or to severity of illness. In general, the secretory responses resembled those seen in infants infected with respiratory syncytial virus.

Enveloped, helical, negative-stranded riboviruses are the principal viral respiratory pathogens of infancy and childhood (5, 13, 14). Parainfluenza viruses are responsible for a wide spectrum of respiratory illnesses ranging from mild rhinitis and bronchitis to severe, potentially life-threatening laryngotracheitis (viral croup) and bronchopneumonia (3, 12, 17, 20, 27). Primary infections usually occur during infancy (19, 28). Reinfections are not uncommon in later life despite preexisting circulating antibody (1, 6). Previous trials of parenterally administered, inactivated parainfluenza viral vaccines in children have shown them to be immunogenic and without paradoxical effects, but not protective (4, 11). Experimental infections in adult human volunteers have indicated that nasal neutralizing activity is associated with immunoglobulin A (IgA) and may have a protective role (23, 25, 26).

The immunological events during and after natural infections with parainfluenza viruses in infants and children are not well defined. Specifically, the secretory antibody response and its role in the natural history of and recovery from croup and other parainfluenza virus-associated illnesses need clarification.

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MATERIALS AND METHODS

Study population. During the 3-month period, 19 September through 13 December 1977, there were 35 isolations of parainfluenza virus types 1 and 2 from patients seen on the pediatric wards or in the Child Care Clinic of Colorado General Hospital. A total of 20 infants and children (12 boys, 8 girls), ranging in age from 7 months to 6 years, were studied. Twelve patients had croup and 15 had fever (>38°C). All but five children were outpatients.

After parental consent was obtained, nasopharyngeal secretion (NPS) specimens were collected by gentle suction (18), initially at the time of the first clinic visit or admission to hospital, and in some cases a week or more after the onset of the monitored illness. A small portion of the early (or acute phase) sample was placed in veal infusion broth, and the remainder was stored at −70°C until processing. Early NPS specimens were available from 19 of the 20 infected patients and serial specimens were available from 11. A total of 35 samples were tested.

Virus isolation and identification. NPS specimens in veal infusion broth were inoculated onto monolayers of primary rhesus monkey kidney (RMK) cells and observed for cytopathogenic effect daily and for hemadsorption (HAd) every 2 to 3 days. Isolates exhibiting HAd were identified by indirect immunofluorescence by using parainfluenza virus type-specific antisera prepared in rabbits.

Processing of NPS specimens. Frozen NPS spec-

imens were thawed rapidly, in some cases diluted with an equal volume of phosphate-buffered saline (pH 7.2), homogenized by vigorous shaking with sterile glass beads, and then centrifuged at 2,000 rpm for 10 min. The slightly cloudy, cell-free supernates were distributed into 0.2-ml portions and stored at −70°C until testing.

Antibody measurement by immunofluorescence technique. An indirect immunofluorescence technique was used to measure class-specific antibody titers of secretion specimens to the homologous and heterologous parainfluenza virus types. HEp-2 cells grown on glass microscope slides were infected with parainfluenza virus type 1 (C-35 strain), type 2 (Greer strain), or type 3 (C-243 strain; all strains were obtained from the American Type Culture Collection), incubated for 24 to 48 h, washed in phosphate-buffered saline, air-dried, then fixed for 10 min in acetone at 4°C. The fixed, infected cells on each slide were divided into eight squares with a moistened, cotton-tipped swab. Fixed, uninfected HEp-2 cells served as controls.

Secretions were pipetted onto the squares, incubated at 37°C for 1 h, and then removed by three successive saline baths. Fluorescein-conjugated goat antiserum to human IgA (Burroughs-Wellcome Co., Research Triangle Park, N.C.), IgG, or IgM (Meloy Laboratories, Inc., Springfield, Va.) was then placed on the slides for 0.5 h and then removed by three additional washes with phosphate-buffered saline. Single lots of conjugate for each immunoglobulin class were used throughout the study.

NPS specimens were initially screened undiluted, and only those showing fluorescence were titrated in twofold increments. All readings were performed under code by one observer (R.Y.). Endpoints were defined as the highest dilution of NPS at which definite fluorescence (rated 1+) was seen.

Virus neutralization tests. Working pools of parainfluenza virus type 1 and type 2 were prepared in primary monkey kidney and in HEp-2 cells, respectively, for use in the neutralization tests.

Neutralizing activity of secretion specimens to parainfluenza virus type 1 was determined by a micromethod (22, 24). Secretions were initially diluted 1:4 in PBS and then tested at twofold increments in duplicate. Samples were mixed with 25 to 50 50% tissue culture infective doses ($TCID_{50}$) of virus, incubated at room temperature (24°C) for 1 h, and then inoculated onto monolayers of secondary RMK cells in microtiter trays. After incubation at 35 to 37°C in a 5% CO_2 atmosphere for 3 days, 0.025 ml of a 1% guinea pig erythrocyte suspension was dispensed into all wells and HAd was recorded. The highest dilution of NPS at which no HAd (complete neutralization) occurred was considered the neutralization titer.

A slight modification of the plaque reduction technique reported by Döhner and Herrmann (7) was employed to measure neutralizing activity to parainfluenza virus type 2. Briefly, duplicate fourfold dilutions of secretion specimens were mixed with 50 to 90 plaque-forming units (PFU) of virus and incubated at room temperature for 1 h before being inoculated onto monolayers of HEp-2 cells grown in plastic trays (Linbro Chemical Co., New Haven, Conn.). After an adsorption period of 1.5 h at 37°C, the inocula were removed and replaced with an overlay of Earle medium containing 5% agamma-calf serum and 0.75% methylcellulose and then incubated at 35 to 37°C for 3 days. HAd plaques were accentuated by staining with 0.025% 3,3'-diaminobenzidine tetrahydrochloride and 0.005% hydrogen peroxide. The neutralizing activity titer was expressed as the reciprocal of the NPS dilution which reduced the number of viral plaques per well by 60%.

Immunoglobulins in NPS. The immunoglobulin levels of secretion specimens were determined by single radial immunodiffusion (18) using a serum IgA standard. The total concentrations of IgA in secretions ranged from 4.6 to 48 mg/100 ml; the mean was 21.7 mg/100 ml. IgA was undetectable (<2 mg/100 ml) in three NPS specimens, and these were excluded from further consideration.

RESULTS

Relationship between neutralizing activity and IgA antibody. There was a marked discordance between nasal neutralizing activity, as determined by $TCID_{50}$ or plaque-reduction, and IgA antibody, as measured by the indirect immunofluorescence technique, to the homologous parainfluenza virus type (Fig. 1). Nearly half (9/19) of the secretion specimens contained neutralizing activity in the absence of detectable IgA antibody. Conversely, two of five and seven of nine specimens from patients infected with parainfluenza virus type 1 and type 2, respectively, had measurable IgA antibody, but lacked neutralizing activity.

Relationship between nasal antibody and infection. None of the 19 early secretion specimens contained detectable IgA, IgM, or IgG antibodies to the homologous virus type (Table 1). With one exception, the acute-phase NPS specimens lacked IgA antibody to the heterologous parainfluenza virus type. On the other hand, neutralizing activity to the homologous virus type was detected commonly (9/19 specimens) in early secretions. There was no correlation between the presence of neutralizing activity in such specimens and the presence or absence of croup.

Serial secretions from four patients infected with parainfluenza virus type 1 and from seven patients infected with type 2 were then examined for changes in fluorescent antibody and neutralizing activity during and after the monitored illness. All 11 patients showed a fourfold or greater titer rise in IgA antibody to the homologous parainfluenza virus type (Table 2). There was no apparent difference in IgA titer rise between patients with and without croup or between the five patients who were younger than 18 months and the six who were older than 18 months. On the other hand, titer rises in

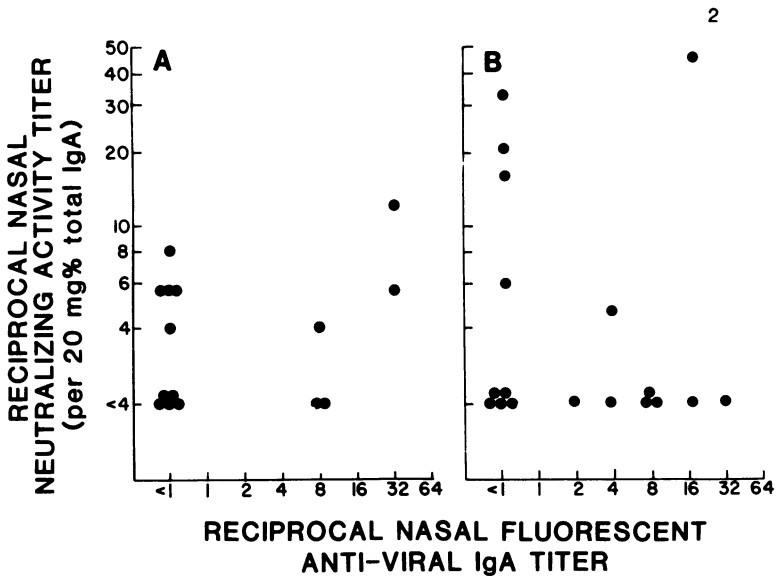

FIG. 1. *Correlation between neutralizing activity and fluorescent IgA antibody to the homologous parainfluenza virus type in individual NPS specimens from patients infected with parainfluenza virus types 1 (A) and 2 (B). Each point represents one specimen for which both tests were performed.*

TABLE 1. *Activity of antibody to the homologous and heterologous parainfluenza virus type in acute-phase NPS specimens*

Infection and no. of patients	No. of patients with acute phase				
	Nasal fluorescent IgA antibody against:			Nasal neutralizing activity against:[a]	
	Parainfluenza virus type 1	Parainfluenza virus type 2	Parainfluenza virus type 3	Parainfluenza virus type 1	Parainfluenza virus type 2
Parainfluenza virus type 1 (10)	0	0	1	5	ND[b]
Parainfluenza virus type 2 (9)	0	0	0	5	4

[a] Neutralizing activity titers equal to or greater than 4, as determined by either plaque or $TCID_{50}$ reduction, were considered significant.

[b] ND, Not done.

neutralizing activity to the homologous virus type were seen in only three patients.

The secretory antibody response to the infecting virus is shown in Fig. 2. IgA antibody was undetectable up to 5 days after the onset of coryza or cough. Antibody appeared at 7 to 10 days and peaked at about 2 weeks. IgA antibody was present in two secretion specimens from which virus was recovered. In three patients, IgA antibody to the homologous virus type was still measurable 8 or more weeks after the onset of the monitored illness.

In contrast, there was no temporal relationship between nasal neutralizing activity and the onset of symptoms or course of illness (Fig. 3); neutralizing activity to the infecting virus type was frequently present in secretion specimens obtained shortly after the onset of respiratory symptoms, but was usually absent when IgA antibody was maximal. Moreover, four secretion samples from children infected with parainfluenza virus type 2 possessed neutralizing activity to both the homologous and heterologous parainfluenza virus type. In addition, one NPS specimen lacked detectable neutralizing activity to the homologous virus type, but contained such activity to the heterologous virus type. The ability of secretions to neutralize respiratory syncytial virus was not tested.

Heterotypic antibody response. IgA antibody to the heterologous parainfluenza virus type was detected in late-convalescent-phase secretion specimens of two patients. One child, a 10-month-old girl with parainfluenza virus type 1 infection, developed an IgA titer of 1:16 to parainfluenza virus type 2 3 months after the

monitored illness. The other patient developed IgA and IgM antibodies to parainfluenza virus type 1 6 weeks after a documented infection with parainfluenza virus type 2. Because of the protracted period (12 and 6 weeks, respectively) from the onset of the monitored illness, it is unclear whether the IgA antibody actually represented a response to a subsequent parainfluenza virus infection, thus indicating a homotypic rather than heterotypic response.

Immunoglobulin classes of antibody to the infecting virus. IgM and/or IgG antibodies to the infecting virus type were detected commonly (82%) in convalescent-phase secretion specimens, but IgA antibody was clearly the predominant class (Table 3).

DISCUSSION

Although specimens from a relatively small number of patients were examined, the data appear to indicate that infants and children naturally infected with parainfluenza virus type 1 or type 2 lack IgA antibody to the homologous virus type in early secretion specimens, that infected patients develop nasal secretory antibody to the homologous virus type, that the capacity of the response does not appear to be related to age or to severity of illness, and that there is a marked discrepancy between neutralizing activity and secretory antibody, as measured by indirect immunofluorescence, to the infecting virus type. In general, these findings resemble those previously demonstrated in respiratory syncytial virus-infected infants younger than 6 months (18).

TABLE 2. *Relationship between secretory response and infection*

Infection	No. of patients		
	with serial secretions	with ≥4× rise in IgA antibody to homologous virus	with ≥4× rise in neutralizing activity to homologous virus
Parainfluenza virus type 1	4	4	2
Parainfluenza virus type 2	7	7	1

An immediate criticism of this study may well be that the immunological status of the patients, as defined by circulating antibody to the infecting parainfluenza virus type, was not known, and that previous infection may have directly or indirectly influenced the local immunological response. Of the 11 patients who had serial NPS specimens, five were younger than 18 months. Only one isolate each of parainfluenza virus type 1 and type 2 were reported by the Virus Diagnostic Laboratory in the 18-month period before the commencement of the study. Therefore, it would appear that these five infants were probably experiencing primary infections. Their secretory responses did not differ qualitatively from those of the six patients older than 18 months who may or may not have had previous experience with the infecting virus type.

After experimental (2, 8, 21, 25) and natural (10) viral respiratory infections, the development of neutralizing activity in NPS has been demonstrated in adults. The neutralizing activity usually peaked 2 to 4 weeks after inoculation or the onset of illness. Smith et al. (25) showed that in adult male volunteers, neutralizing activity in preinfection nasal secretions was a far better index of host resistance to intranasal challenge by parainfluenza virus type 1 than neutralizing activity in serum, which was detectable in all of the men studied. Volunteers with low or undetectable nasal neutralizing activity invariably became infected. This neutralizing effect was

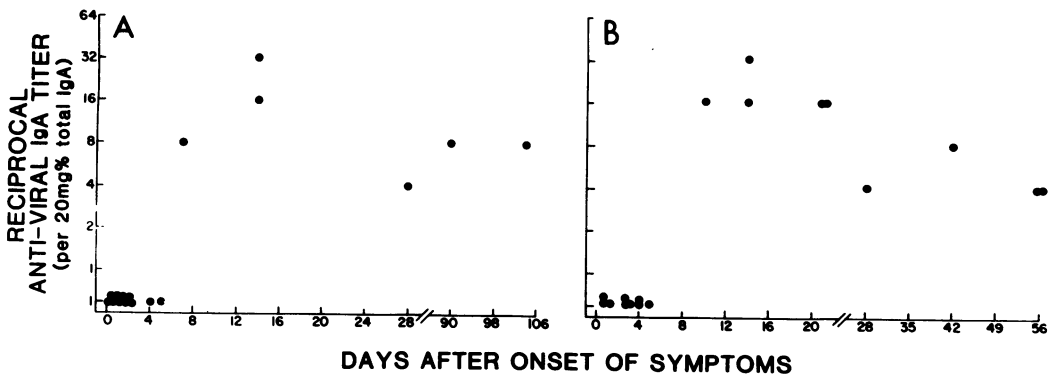

FIG. 2. *Fluorescent IgA antibody to the homologous parainfluenza virus type in NPS from infants and children infected with parainfluenza virus types 1 (A) and 2 (B). The data are presented in relation to the onset of symptoms and have been adjusted to 20 mg of total IgA per 100 ml.*

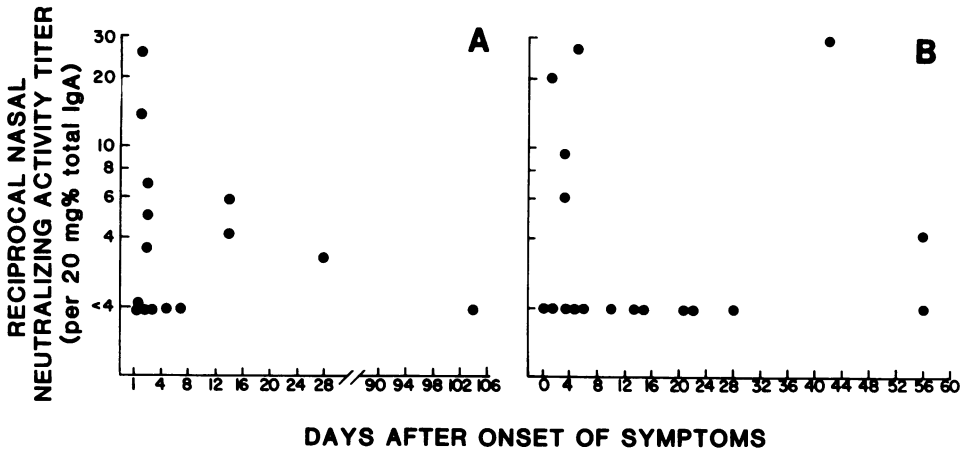

FIG. 3. *Neutralizing activity in NPS specimens from infants and children infected with parainfluenza virus types 1 (A) and 2 (B). The data have been adjusted to 20 mg of total IgA per 100 ml and are presented in relation to the onset of clinical illness.*

TABLE 3. *Immunoglobulin classes of antibody to parainfluenza virus types 1 and 2 in NPS*

Immunoglobulin class of antibody to homologous virus type	No. of secretions with maximal antibody to homologous virus type	
	Parainfluenza type 1	Parainfluenza type 2
IgA	3	6
IgG	0	1
IgM	0	0
IgA=IgG=IgM[a]	1	0

[a] The level of antibody of the three classes was the same and maximal. Only secretions in which antibody of at least one class (as determined by indirect immunofluorescence technique) was detected are included.

associated with the IgA portion of fractionated NPS specimens (23). The protective role of nasal neutralizing activity has also been inferred from studies in adults experimentally infected with parainfluenza virus type 2 (26) and other respiratory viruses.

In this and a previous study (18), neutralizing activity was frequently detected in early NPS specimens and titer rises in neutralizing activity did not usually occur. In addition, there was a striking discrepancy between neutralizing activity and IgA antibody to the infecting virus type: at one extreme, neutralizing activity was present in the absence of detectable IgA antibody, and at the other, no neutralizing activity was detectable when IgA antibody was maximal. The discordance between neutralizing activity and IgA antibody may represent a general phenomenon of the secretory response to viral respiratory infections in infants and children. Alternatively, as reported recently for respiratory syncytial virus (16), antigenic change(s) in the prevalent parainfluenza virus strains might have resulted in the failure of postinfection antibody to neutralize standard virus strains.

The precise mechanisms of recovery from natural infection with parainfluenza viruses are unknown. It is uncertain whether secretory IgA antibody has a curative role. Recently, interferon has been demonstrated in acute-phase nasal secretion specimens of children infected with parainfluenza virus type 1 (15). The occurrence of persistent and fatal, disseminated myxovirus and paramyxovirus infections in children with immunodeficiency syndromes characterized by defects in cell-mediated immunity (9) suggests that local cell-mediated immunological responses may be more important in the recovery process.

Finally, the data do not allow any definite conclusions about pathogenesis. However, the absence of IgA antibody to the homologous virus type in early NPS specimens argues against a previous sensitizing infection. Moreover, antibody to the heterologous parainfluenza virus type was found in only one secretion (Table 1). This implies that secretion antibody produced by prior infection with heterologous parainfluenza virus types is probably not involved in the pathogenesis of croup. The presence of both culturable virus and IgA antibody in NPS specimens of two patients supports the hypothesis that disease probably results from the concurrent events of virus replication and the immunological response of the host.

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